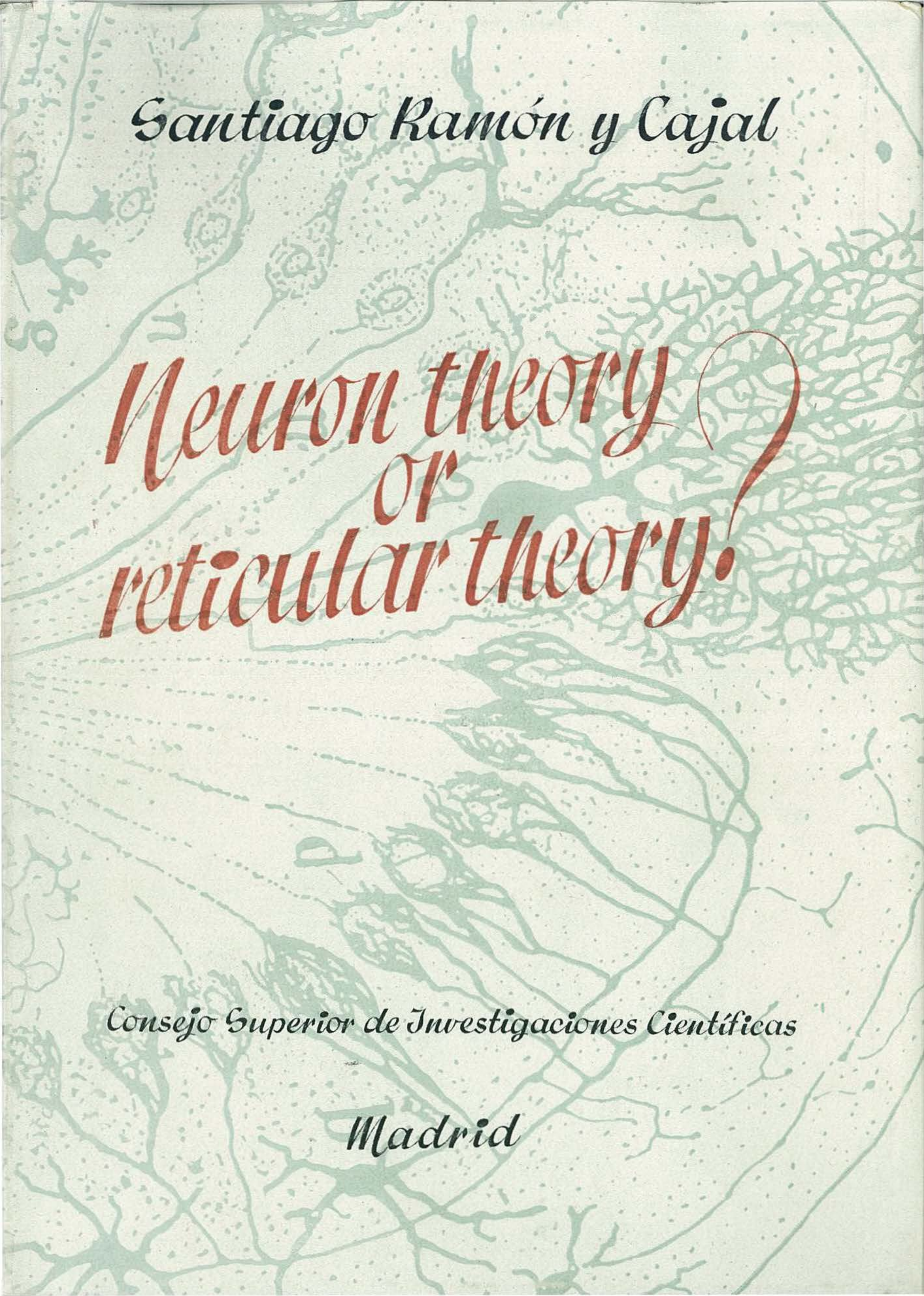

Santiago Ramón y Cajal

Neuron theory or reticular theory?

Consejo Superior de Investigaciones Científicas

Madrid

NEURON THEORY OR RETICULAR THEORY?

NEURON THEORY OR RETICULAR THEORY?

Objective Evidence of the Anatomical Unity of Nerve Cells

by

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Translated

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TABLE OF CONTENTS

Pages.

Ramón y Cajal, an appreciation… … … … … … … … … … … … … … … IX
Preface… XI
Translators' Note… … … … … … … … … … … … … … … … … … XV

PART ONE

CHAPTER I

Historical notes about the neuron theory … … … … … … … … … … 3

CHAPTER II

Histological proofs… … … … … … … … … … … … … … … … … … … 10
Varieties of Synapses… … … … … … … … … … … … … … … … 10
The Neuronal Membrane … … … … … … … … … … … … … … … 12

CHAPTER III

I. Axo-somatic connections by means of nerve baskets rich in fibres ("nests" or "baskets" of the cerebellum, etc.)… … … … 16
Objections to my interpretations of the baskets and brushes… … … 21
A. Objections of Golgi and his School… … … … … … … … … 21
B. Objection of Bielschowsky based on his neurofibrillar preparations… … … … … … … … … … … … … … … … 23
II. Axo-somatic connections by means of baskets or nests poor in axonal branches… … … … … … … … … … … … … … … … 25

CHAPTER IV

III. Nests or chalices of Held in the nucleus of the trapezoid body. 27
Critical observations of Held and doubts of Bielschowsky… … … … 30
IV. Axo-somatic connections an the ventral cochlear nucleus… … 38

Pages.

CHAPTER V

V. PERIPHERAL TERMINATIONS OF THE COCHLEAR NERVE IN THE ORGAN OF CORTI. TERMINATIONS OF THE VESTIBULAR NERVE... 39
  A. Terminations of the cochlear nerve... 39
  B. Peripheral terminations of the vestibular nerve... 44

CHAPTER VI

VI. AXO-SOMATIC CONNECTIONS BY MEANS OF THICK TERMINAL TUBERCLES WITH OR WITHOUT PERICELLULAR PROJECTIONS (RETINA). TUBERCLES OF THE VESTIBULAR NERVE IN THE LATERAL NUCLEUS OF THE MEDULLA IN BIRDS AND FISHES. BULBS OF HELD. "ENDFÜSSEN" OF THE MOTOR NERVES... 51
  A. Axo-somatic connections in the retina... 51
  B. Tubercles of the vestibular nerve in the lateral nucleus of the medulla in birds, reptiles and fishes... 53
  C. Axo-somatic connections by means of large, loose nests from which arise bulbs and terminal feet ("Endfüssen" of Held)... 55
My new observations on the "Endfüssen"... 60
Opposition of Held's school and doubts of Bielschowsky concerning the independence of the "Endfüssen" and rings... 62

CHAPTER VII

VII. CLIMBING FIBERS OR LONGITUDINAL AXO-DENDRITIC CONNECTIONS. AXO-DENDRITIC CONNECTIONS BY MEANS OF GEARS... 69
Climbing fibers of the sympathetic ganglia: thicket-like terminations. 73
VIII. AXO-DENDRITIC CONNECTIONS BY GEARING (MOSSY FIBERS, INSECT RETINA, ETC.)... 75
  A. Gearing between granule and mossy fibers... 75
  B. Axo-dendritic connections by gearing in Ammon's horn... ... 79
  C. Gearing connections in invertebrates... 81

CHAPTER VIII

IX. CRUCIFORM AXO-DENDRITIC CONNECTIONS OF GREAT LENGTH. MOLECULAR LAYER OF THE CEREBELLUM AND THE INTERPEDUNCULAR NUCLEUS... ... 84
  A. Cruciform connections in the cerebellum. Relationship by means of plane, parallel arborizations... 84
  B. Axo-dendritic connections by means of plane, parallel plexuses. 86

Pages.

CHAPTER IX

X. TERMINATIONS UPON CIRCUMSCRIBED CELLULAR PLEIADES. CONNECTIONS IN THE THALAMUS. COMPLEX CONNECTIONS IN THE CEREBRAL CORTEX. 88
  A. Terminal arborizations in the thalamus... 88
    a) Sensory terminations... 88
    b) Terminations in the habenular ganglion... 90
    c) External geniculate body... 90
  B. Complex terminations in the mammalian cerebral cortex... 91
    a) Synapses between the extremely complex, diffuse nerve plexuses and the bodies and shafts of the cerebral pyramids... 94
    b) Terminations of centripetal fibers from the thalamus in the sensory cortex... 100
    c) Terminations of the neurons of short axon... 101
    d) Axonal terminations of Martinotti neurons and similar types... 103

CHAPTER X

XI. APPARENT OBJECTIONS TO THE NEURON THEORY BASED ON RECENT STUDIES OF THE SENSORY AND MOTOR END-ORGANS... 104
  A. Motor terminations and Boeke's periterminal network... 104
  B. Terminations in smooth muscle fibers... 109
  C. The periterminal network of Boeke in the sensory organs... ... 110
  D. My recent investigations on the organs of Grandry and Herbst. 113

CHAPTER XI

XII. THE LATEST CONCEPTION OF HELD ON THE STRUCTURE OF THE GRAY SUBSTANCE... 117

PART TWO

CHAPTER XII

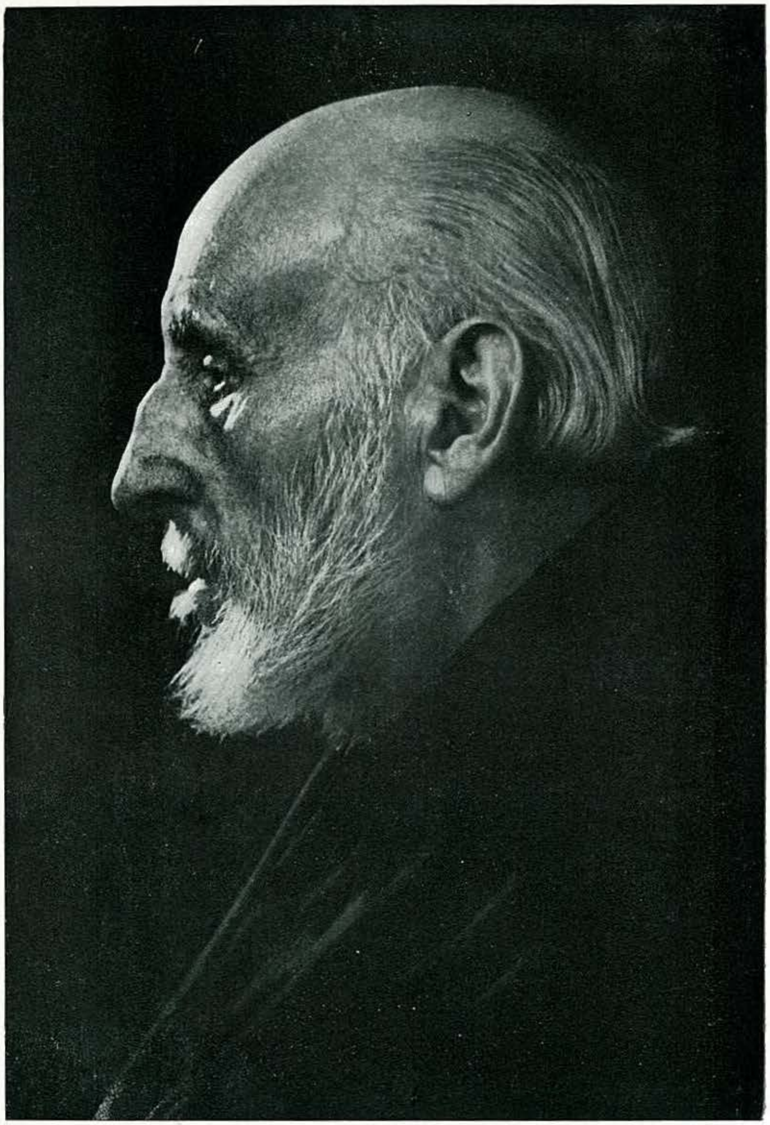
I. NEUROGENETIC UNITY AS A PROOF OF THE NEURON THEORY. FREE GROWTH OF THE AXONS IN TISSUE CULTURE. ERROR OF THE SUPPOSED INITIAL ENSHEATHMENT IN THE COURSE OF THE EMBRYONIC AXONS. FREE GROWTH OF THE NEWLY-FORMED FIBERS IN THE SCAR OF THE SECTIONED NERVE... 127

Pages.

A. Neurogenetic unity of the neurons and axons... 127
B. The theory of autoregeneration and ensheathment in the regrowth of sectioned nerves... 133
C. Regeneration in the nervous centers... 137

CHAPTER XIII

II. ANATOMICOPATHOLOGICAL FACTS FAVORABLE TO THE NEURON THEORY. PERSISTENCE OF THE BASKETS AND OTHER PERICELLULAR TERMINATIONS, ETC. 137
CONCLUSION... 143



S. Ramón Cajal

Ramón y Cajal, an appreciation

He was confined to bed with a cold, dressed in a gray sweater and cap, when I last saw Don Santiago Ramón y Cajal. The manuscript of this book —«Neuronismo o Reticularismo. The Objective Proofs of the Unity of Nerve Cells»— lay in his lap. As we came into the room, Pío Del Río-Hortega and I, he put down the goose quill with which he had been writing and smiled a courteous welcome. The wall adjacent to his impatient right hand was spattered with ink.

The master was then in his eightieth year, alone in his home in Madrid, for Señora Cajal had passed on. He was feeble, and deafness was closing doors to the world around him. But within him there burned the boundless enthusiasm of the born explorer and his eyes blazed at us through shaggy brows as he talked.

He was a many sided genius, a man who would have been a leader in any land, at any time.

Ramón y Cajal! We salute «the pilgrim soul in you»— great Spaniard, scientist, prophet.

WILDER PENFIELD, O. M., F. R. S.

Preface

Toward the end of his long scientific career Professor Ramón y Cajal reassembled and re-evaluated his evidence for the integrity of the individual neuron and for his concept of the dynamic interdependence and interrelations of nerve cells at the synapses in a monograph entitled: «¿Neuronismo o Reticularismo?» which appeared after his death. A French translation of this monograph was published in the Travaux du Laboratoire de Recherches biologiques de l'Université de Madrid (v. 29, pp. 1-37) under the title «Les preuves objectives de l'unité anatomique des cellules nerveuses». The somewhat modified German version of the article was incorporated under Ramón y Cajal's name in volume I (pp. 887-994) of the Bumke and Foerster 'Handbuch der Neurologie' (Berlin, Springer, 1935). The present translation is based on the Spanish edition.

That the English edition of this last book of Ramón y Cajal should have been prepared for publication during 1952 is most fitting since this year marked the centenary of his birth. In fact this edition was a part of a general program for paying tribute to this great Spanish scientist —this winner of the coveted Nobel Prize in Medicine and Physiology— on his hundredth anniversary. In his own country, Spain, various ceremonies and a special scientific meeting honored the occasion and many scientific journals in Western Europe and the Americas joined in the general acclaim.

The magnificent technical genius of Ramón y Cajal, the amazing keennes of his observations, the incredible amount of histologic details concerning the structure of the nerve cells which he was able not only to see and record but also to interpret, his superb ability to organize this detail into meaningful patterns and his understanding of this finer histologic structure under special operative or pathologic conditions have given him a unique and probably unequalled position among research workers in his own and closely related fields. The mere mention of a few of his contributions to the present concept of the nerve cell may serve to illustrate their basic importance. In 1888 his description of basket formations about the Purkinje cell bodies supplied the first direct evidence of actual contact between nerve cells. His account, again in the same year, of the intimate contact existing between climbing fibers and the dendrites of Purkinje cells dispelled Golgi's theory that the dendrites were merely elaborate nutritional devices. These facts provided the foundation for Cajal's concept of the dynamic polarization of the nerve cell. Substantially, then, he had fathered the concept of the integrity of the individual nerve cell and of the arrangement of the nerve cells into functional neuron arcs. Waldeyer, in a review summarizing the ideas of Cajal, styled this concept the «neuron theory» and to this day the parent and the sponsor have frequently been confused.

In this last book Ramón y Cajal discussed the neuron theory, the history of its development and the evidence on which it is based. He supplemented the facts obtainable by detailed microscopic study of the neurons —their cell bodies, their processes, their synapses, their interrelations— by evidence afforded from his work on the degeneration *and regeneration of nerves. This material is enriched further by certain physiological and pathological data. The literature favoring his views and also that opposed to these ideas is handled courteously and skillfully. The illustrations are beautifull.*

The two men who were chosen to prepare this translation have been particularly well suited to their task. Rev. Dr. M. Ubeda Purkiss, who at the present time is actively engaged in research at the Cajal Institute, has access to the original Cajal material and association with scholars, excellent scientists in their own right, who were trained under the master. His several years of residence in the United States gave him a good command of English and led to a close acquaintanceship with his co-worker Dr. Fox, which has made the translation more feasible. Dr. Clement Fox, a Professor of Anatomy at Marquette University School of Medicine, has an enviable reputation in research in neuroanatomical fields. His admiration for the work of Ramón y Cajal is well-known and has led him to give considerable time to the publications of this great observer and to the application of the Cajal technical methods to his own research problems.

The English-speaking scientific world is very grateful to these translators for making available this excellent summary of the great contributions of Professor Ramón y Cajal to our knowledge of the finer structure of the nervous system. The whole purpose of the book is revealed in its author's own words which, in translation, are: «to describe briefly what I have seen during 50 years of work, and what any investigator can verify for himself».

ELIZABETH C. CROSBY.

The University of Michigan.
January 5, 1953

Translators' Note

In his last monograph Ramón y Cajal, to use his own words, proposed, «to describe briefly what I have seen during fifty years of work and what any investigator can verify for himself». It is a summary that he was anxious to make at the end of his long career for his work played the major role in the development of the modern concept of the nerve cell. The monograph is a survey of the evidence for and against the neuron theory. But it is more than that, it is almost an autobiographical account of his scientific life. Thus it seemed most fitting to put it into English at the time the scientific world is taking note of his centennial. It has appeared in Spanish, French and German editions. The present translation was made from «¿Neuronismo o Reticularismo?», the Spanish version which used the first person, singular; the more formal «we» was substituted in the French and German versions. We have felt that the first person singular was more in keeping with the intent of the author who, at the age of eighty, here summarized his life's work. We hope that we have succeeded in preserving the informal vigor of the original. Perhaps now, one century after his birth, the master may be accorded the privilege of saying. «This is what I have seen».

The first draft of this translation was read and notably improved by John E. Dooley, student of the Medical School of Marquette University and Research Fellow of the Cajal Institute during the year of 1952. To him we owe a debt of gratitude.

Neuron Theory or Reticular Theory?

Objective Evidence of the Anatomical Unity of Nerve Cells

by

S. Ramón y Cajal

Despite the wealth of objective evidence favoring the doctrine of the individuality of the constituent elements of the gray substance, now and then we observe new interest in the reticular theory. This is particularly true since APATHY's apparent demonstration that a continuous net exists in the ganglia of invertebrates between the neurofibrils of the *Püncksubtanz.* Today, once again, we are witnessing a rebirth of this theory of continuity, thanks to the tireless activity of HELD and his disciples.

I am not at all surprised at this return to GERLACH's old doctrine of nerve nets. It is necessary to realize that for certain minds the reticular theory offers a most attractive and convenient explanation. Among other physiological advantages it would offer the inestimable one of explaining in a simple manner the propagation of the nerve impulse from one neuron to another and its diffusion throughout the gray substance in a number of directions.

The important thing here is not to ponder the theoretical simplicity and facility (more apparent than real) of a theory but rather to evaluate to what extent it conforms with well-known, demonstrable facts.

Rather than being a sterile attempt to raise a new controversy, the present paper will be a concise exposition of observations which refute the ideas of APATHY, BETHE and HELD. I propose to describe briefly *what I have seen* during fifty years of work and what any investigator can verify for himself. It is not based on this or that nerve cell (which may be of abnormal type or very badly fixed) but rather on millions of neurons strongly stained by different methods of impregnation.

I am also moved by a desire for clarity. Although defended by its more authoritative and brilliant patrons, the reticular theory appears obscure and contradictory. Every anti-neuronist adheres to a personal doctrine which has few points of similarity with that of his companions. What is worse, all these opinions have little in common with the positive data brought forth by the physiology, pathology, ontogeny and phylogeny of the nervous system. On the contrary, the basis of the neuron theory is strengthened and fortified by evidence from neurogeny, nerve regeneration, physiology, pathology, etc.

It is obvious that techniques of the future may unveil new data favoring the reticular theory or other theories. A little improvement in method or a histological discovery of general importance may force us to modify our conclusions. But for the time being such a revision seems neither imminent nor probable. We may, therefore, adopt without reservation the conclusive doctrine of HIS, FOREL and KÖLLIKER because it is based upon innumerable and concordant facts observed in the nervous system of vertebrates and invertebrates.

For the sake of clarity I shall follow this order:

Part one.

A. History of the neuron theory.

B. The mass of careful observations upon which this theory is based, including modern doubts and objections to it.

Part two.

C. Irrefutable evidence favoring the neuron theory.

D. Favorable data from the process of nerve regeneration and the method of nerve culture.

E. In brief, because of the limited extent of this presentation, a few arguments obtained from physiology and pathology.

PART ONE

CHAPTER I

HISTORICAL NOTES ABOUT THE NEURON THEORY

With good reason the illustrious authors, W. HIS (1886-1890) and FOREL (1887), have been credited with the enlightened idea that the expansions of nerve cells end freely in the gray substance just as they do in the peripheral sense organs. Professor WALDEYER (1) in Germany was the main advocate of this theory, together with KÖLLIKER, SCHIEFFERDECKER, etc. In France, where the most active proponent was MATHIAS DUVAL, the doctrine was received enthusiastically thanks to my evidence. Finally, the new doctrine was presented with great vigor in Italy by TANZI and LUGARO.

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(1) Professor WALDEYER, to whom poorly informed persons attribute the neuron theory, supported it with the prestige of his authority but did not contribute a single personal observation. He limited himself to a short, brilliant exposition (1891) of the objective proofs, adduced by HIS, KÖLLIKER, RETZIUS, VAN GEHUCHTEN and myself, and he invented the fortunate term of *neuron.* Popular explanations of the *entire theory,* illustrated with very expressive diagrams and pictures, favoring the neuronal doctrine, were published shortly after my writings by VAN GEHUCHTEN (1891), v. LENHOSSÉK (1891), KUFFER (1892), CHARPY (1892), DAGONET (1892), AZOULAY (1893), BAKER (1893), TANZI (1893), E. A. SCHAFFER (1893), HIS (1893), HELD (1893) —who made a resumé of my public lectures of Barcelona—, GUSTAV RETZIUS (1896), etc. Besides those of WALDEYER, synthetic descriptions, sometimes with schematic plans, were produced by DÉJERINE (1805), M. HEIDENHAIN, P. SCHIEFFERDECKER and other scholars Appropriate as it is to mention the works of these illustrious histologists, we must not overlook the great VERWORN (1900), the eminent German physiologist, and the no less renowned KÖLLIKER (1900), who generously *accepted* my ideas in his numerous publications, enlarging upon them with new illustrations and discoveries. For my part, I presented to the press various controversial articles explaining the doctrine. Of these I mention (apart from my complete book): *El Renacimiento de la doctrina neuronal,* Barcelona, 1907. Die Histogenetischen Beweise der Neuronentheorie von HIS und FOREL. *Anat. Anz.* XXX Bd., 1907; L'hypothèse de la continuité d'Apathy. Reponse aux objections de cet auteur contre la théorie neuronale. *Travaux du Lab. de rech. biol.,* VI, 1908 y *Anat. Anz.,* XXXIII Bd., 1908; Nouvelles observations sur l'évolution des neuroblastes avec quelques remarques sur l'hypothèse neurogénetique de HENSEL-HELD. *Anat. Anz.,* XXXII, 1908.

Collecting the suggestions scattered in the various papers of His, based on his valuable observations on the neurogenesis of man, I wrote, in 1899 (Spanish Edition of my *Histology of the Nervous System)*, more of less the following: "His affirms that the embryonic axons represent the continuation of the first appendage which grows from the neuroblast; therefore, it is necessary to admit that each fiber or *neurit* should grow by extension of its free endings during a very long period of its evolution. There is no reason to think that this process may be modified later, either by the axons or by the protoplasmic expansions. Furthermore, for many years we have had knowledge of a group of free peripheral endings, for example, the free terminations in the cornea, tactile corpuscles of PACINI, MEISSNER and KRAUSE, the motor plates, etc., in all of which His noted that the terminal nerve branches end in a free ending and not by anastomotic arborizations."

According to His the same principle could be applied to the free ending sensory ganglia (1). Incidentally, despite the inadequate methods employed, this investigator discovered the interesting fact that to grow progressively into monopolar cells, the sensory cells originally adopt a bipolar form (as I confirmed in 1890).

For his part FOREL (1887), who had seen some sections made by the GOLGI method, advocated almost simultaneously with His the autonomy of nerve cells or neurons. He based this conclusion above all on GUDDEN's experiments of pulling out axons, which caused only the atrophy of the nerve cells of origin (the trophic centers of DEITERS) (2).

This conception of His and FOREL, based both on direct observations and on very ingenious and accurate generalizations, represented a strong reaction against the reticular theories of GERLACH and GOLGI which at the time dominated the science of neurology.

Very special mention is deserved by the eminent Italian investigator

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(1) HIS: Die Neuroblasten und deren Entstehung im embryonalen Mark, *Abh. der math. physik. Klasse der Köningl. Sachs. Geselsch. d. Wissensch.*, Bd. XV, 1889.

See also: Zur Geschichte des menschlichen Rückenmarks & der Nervenwurzeln, October 1886.

Ibid: Über den Aufbau unseres Nervensystems. *Verhandlungen* etc., 1893.

(2) FOREL (A.): Einige hirnanatomische Betrachtungen & Ergebnisse. *Arch. f. Psych. & Nervenheilkunde.* Bd. 18, 1887.

GOLGI who, despite being a confirmed reticularist, brought forth important evidence favoring the doctrine of contiguity. Armed with two very valuable methods of impregnation (silver chromate and bichloride of mercury), the scholar of Pavia (1873-1886), besides discovering many valuable data on the morphology of neurons and nerve collaterals, contributed to the neuron theory the fundamental fact, revolutionary at the time, that the dendrites course independently through the gray substance and end by free terminations. The very thick and complex net of protoplasmic arborizations which fill the gray substance with marked regional differentiation, was carefully studied by this scientist. What a pity that GOLGI assumed without proof that it lacks the power of conductivity! In the great office of mental life these expansions could assume merely a trophic role! The propagation of the nerve impulse would have as an exclusive pathway a very fine and intricate net spread throughout the gray substance and formed: (a) by the anastomosis of the nerve collaterals of the Type I cells (motor); (b) by the axonal arborizations of the Type II cells (sensory type, which correspond to that class I later called "cells of short axon"); and, finally, (c) by the central ramifications of the axon of the corpuscles of the sensory ganglia (1).

It is a regretable truth that almost no one can be entirely free from the traditions and fashions of his day. Despite his admirable originality GOLGI was badly influenced by GERLACH's doctrine of diffuse interstitial nets. At any rate the influence of GOLGI's theories, particularly in Italy, was considerable. His most eager disciples were L. SALA, FUSARI, MONDINO, VERATTI, MARTINOTTI, etc. He also had enthusiastic followers in foreign countries, although his opponents were most numerous. In Italy, for example, he did not persuade such gifted and clear-thinking investigators as TANZI, LUGARO, and others, nor even his nephew, assistant, and successor at Pavia, the talented and short lived PERRONCITO (2).

As a distant echo of GERLACH's theory the Russian histologist

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(1) See, among the many other works concerning the nervous system, GOLGI's major effort: *Sulla minuta anatomia degli organi centrali del sistema nervoso.* Milano, 1885, and also his *Opera Omnia* published in Italian and German and well known by everyone. Later I shall refer to other works by him.

(2) Died following a disease contracted during the world war.

DOGIEL advanced a modified reticular theory (1891-1893) based upon the accidental union of neurons and nerve fibers caused by the fixatives of EHRLICH's vital staining. He described thick protoplasmic interdendritic bridges in the retina.

Despite the wanderings in the interpretations of DOGIEL and some of his disciples, their work was graced with good fortune because many of their results with the methylene blue method on the sympathetic system, the sensory ganglia, the retina, the cerebellum and in the peripheral nerve endings, are in perfect accord with the neuron theory. In the later years of his life the Russian professor approached closer and closer to this theory. Perhaps my findings with the EHRLICH and GOLGI methods on the retina, sympathetic system and cerebellum may have contributed in some way to DOGIEL's happy doctrinal evolution, destroying among other prejudices that old confining one of anastomoses between thick dendrites.

For the sake of brevity, and considering that I shall speak about them later, I omit here an account of my contributions to the demonstration of the HIS hypothesis. I should like to mention here the curious fact that my ideas were formulated in 1888 and later in 1889 and 1890 without knowledge of the work and conclusions of HIS and FOREL. Only later (1890), thanks to the kindness of the professor of Leipzig, I read the HIS embryological papers which were written previous to my investigations. Due to my lack of bibliographical material I only quoted him in my first neurogenetic exploration of the spinal cord (1890). At any rate this coincidence in thought on the part of the leading workers in the field, without any oral or written collaboration, constitutes the best moral encouragement and the strongest guaranty of the validity of the adopted interpretation.

With pleasure I should add the name of H. HELD to the list of collaborators to the neuron theory, because during the years of 1893 and following, he contributed three important facts: the chalices of the trapezoid body nucleus, the pericellular plexuses of the cochlear nerve in the ventral acoustic ganglion, and later, using his own methods, the terminal bulbs of the spinal cord's sensory fibers (*Endfüssen)*. With such findings HELD added to the strength of the theory of contiguity, although he somehow modified it later with the hypothesis of *encrustation* in perfect accord with the HIS concept. But unfortunately HELD later accepted the opinion of the group defending the theory of continuity, especially when he thought he had found evidence for his newest position in certain doubtful images obtained by the neurofibrillar method and the GOLGI technique. I shall speak presently of the objections against the neuron theory presented by HELD, BIELSCHOWSKY and others, and shall speak also of the delusions and difficulties menacing the most cautious neurologist when some rare and seldom found microscopic images are interpreted too literally because of over enthusiasm.

And now I devote a word to a wise precursor of the neuron theory. I believe that MAX SCHÜLTZE was such a man, for although today he is almost entirely forgotten by the masters, he discovered the neurofibrils and other facts. In his well known article in *Stricker's Handbuch* (1871) we find positive observations as well as some criticisms full of wisdom and prudence. In all these observations the doctrine of discontinuity is a tacit postulate. In the drawings with which he illustrated his paper we look in vain for even a single anastomosis or a sign suggestive of GERLACH's syncytium (1). Moreover, in the text there is evidence as conspicuous as this: "Die feinen Faserchen (Primitivfibrillen) welche aus den Verästelungen hervorgehen entziehen sich sehr bald der Beobachtungen. Ihr endliches Schicksal ist unbekannt." Concerning the anastomosis by means of thick dendrites, without an absolute denial of their existence, he says: "Die sorgfältigsten Isolierungsversuche von Deiters haben nur negative Resultate ergeben. Ebenso ist es mir bei vielen bezüeglichen Versuchen an dem zum Studium der Ganglienzellen unübertrefflich geeigneten elektrischen Lappen des Gehirns zur Zitterochengegangen, obgleich Rudolf Wagner hier früher deutliche Anastomosen erkannt zu haben angibt, habe ich mittles besserer Isolierungsmethoden kein Beispiel einer solchen finden können." Neither does he see anastomoses in the peripheral nerve endings.

This exquisite viewpoint, which we would like to see shared today by some famous leaders in neurology, guarded MAX SCHÜLTZE from falling into the errors and fantasies of HARLESS, LIEBERKÜHN, WAGNER, ARNOLD, FROHMANN, BEALE, JOLLY, MEYNERT (so meritorious in other concepts), ARNDT, BESSER and many more.

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(1) See figure 28, referring to the anterior horn of the spinal cord (Allgemeines zur den Strukturelementen des Nervensystems. *Stricker's Handbuch*, 1871).

The brilliant discoveries of HIS and FOREL and the work of GOLGI lack two important findings: a clear and irrefutable demonstration of the means of axonal termination in nerve centers, and the true relationships of nerve ramifications with neurons.

HIS, who used hematoxylin applied preferably in the human embryo, observed the neuroblasts perfectly, as I mentioned above, but he did not reach an understanding of intra- and extramedullary axonal terminations. His embryonic neurites appear to end in probe-like tips, whereas in reality they terminate by means of clubs or bristly, thorny thickenings and rudimentary appendages, as MICHAEL VON LENHOSSÉK (1890) and I have shown.

In reality, the first discovery of the manner of termination of an adult axon occurred in 1888 in the avian cerebellum (1), and the first time that the tip of an embryonic axon in the growth process was seen was in 1890 in the chick embryo in the third day of incubation (CAJAL (2) and VON LENHOSSÉK) (3). Later on I was able to confirm this free termination of neurites with their pericellular apparatus in a large number of organs (cerebellum, spinal cord, AMMON'S horn, olfactory bulb, retina, thalamus, sympathetic trunk, optic lobe, etc.).

Perhaps it will not be without interest to quote here the words with which I fought the reticular theory in 1888 and 1889: "We have made careful investigations of the course and connections of nerve fibers in the cerebral and cerebellar convolutions of man, monkey, dog, etc., and we have never seen an anastomosis between the ramifications of two different protoplasmic expansions, nor have we observed them between the filaments emanating from the same expansions of DEITERS; the fibers intermingle in a most complex manner, producing a thick and intricate plexus but never a net. The observations which we have just explained, concerning the structure of the avian cerebellum, also support this viewpoint; it could be said that each element is an absolutely autonomous physiological canton"

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(1) Estructura de los centros nerviosos de las aves. *Rev. trim. de Histología normal y patológica,* 1 de mayo de 1888, Barcelona, y Morfología y conexiones de los elementos nerviosos de las aves. See also, mayo de 1888, Barcelona.

(2) CAJAL: *Anat. Anzeiger*. Bd. V, 1890.

(3) LENHOSSÉK: *Vernandl. d. X internat. Congrès zu Berlin.* Bd. II, 1890.

I referred here especially to the brush- or tassel-like endings around the PURKINJE cells which I observed for the first time in the hen and dove. This arrangement was confirmed in my *Memoir* of 1889 where I studied particularly the mammalian cerebellum (1), strengthening the neuron theory and enlarging upon its objective foundation.

In another paper of 1888 I described the retinal neurons as independent units, refuting the hypothesis of anastomosis held by DOGIEL and founded upon observations by EHRLICH's method. I said: "I could not observe the nest described by DOGIEL among the inferior arborizations of the bipolar cells, and for this reason I am inclined to think that this author took as anastomoses frequent cases of superimpositions and contacts among the endings of fibers emanating from the different bipolar elements. Perhaps the same easy mistake can explain TARTUFERI's thesis of a supposed anastomotic structure in the horizontal net of the inner zone of the retinal internal reticular stratum" (2).

Soon afterwards there arrived in the recently clarified field a group of enthusiastic workers who confirmed my findings by illustrating and elaborating notably the doctrine of HIS, enriching it with a wealth of evidence of priceless value.

The great KÖLLIKER (3) initiated these explorations with many monographs, among which are conspicuous those on the cerebellum, spinal cord and cerebrum. VON LENHOSSÉK (1892) (4) followed with various interesting contributions on the spinal cord and the nerve chain of *Lumbricus,* etc. Professor RETZIUS of Stockholm (1), with his cleverness and insurmountable patience, continued the work as author of many monumental papers on the nervous system of vertebrates and invertebrates; and almost contemporaneously, or a little after, the problem of the interneuronal connections was studied by van GEHUCHTEN of Louvain (2), EDINGER, P. RAMÓN, LUGARO, FALCONE, NAGEOTTE, MARINESCO, ILLERA, LAVILLA, ATHIAS, BLANES, TELLO, AZOULAY, CALLEJA and many other investigators too numerous to mention. The extremely long bibliography of the defenders of the neuron theory will follow later according to the demands of textual clarity. Here I cite only the first monographs in the long process of confirmation and elaborations of the HIS' doctrine.

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(1) Sur l'origine et la direction des prolongations nerveuses de la couche moléculaire du cervelet. *Inter. Monatschr. f. Anat. u. Physiol.,* Bd. VI, 1889, (Here the nests or baskets of the Purkinje cells in birds and mammals are studied, enlarging upon my original observations.)

(2) Sur la morphologie et les conexions des élements de la rétine des oiseaux. *Anat. Anzeiger,* núm. 4, 1889.

(3) KÖLLIKER: Des Kleinhirn. *Zeitschr. f. wissenschaftl.* Zoologie. Bd. XLIX. 1890.

Also: Ueber der feineren Bau des Rückenmarkes. *Sitz. d. Würzb. phys-med. Gesellsch. 8 Maerz,* 1890.

(4) V. LENHOSSÉK: Der feinere Bau der Nervensystems im Lichte neuester Forschungen (1895). See his important investigations on the *Lumbricus. Arch. f. mikrosk. Anat.* Bd. XXX, 1892, and other valuable writtings on the posterior roots, etc.

CHAPTER II

HISTOLOGICAL PROOFS

Varieties of Synapses. The Cellular Membrane

I have already briefly exposed the historical background of the theory of discontinuity and now it is time to bring forth the very clear and irrefutable facts upon which it is based. I shall not summarize here all of the well known objective arguments - this would oblige me to write an extensive book and, moreover, a superfluous one, for the proofs of the doctrine of HIS and FOREL are collected in all of my books and papers, not to mention the very numerous and conclusive proofs presented by distinguished scientists. Here I shall study exclusively the arrangement of interneuronal connections which seem to me most expressive and unequivocal. For better order in the exposition it is well to draw up a classification. Almost all of the relations by contact which are known up to the present day can be summarized in the following groups:

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(1) RETZIUS: *Biol. Untersuchungen,* 1892, Stockholm, & *Neue Folge,* IV. Die nervösen Elemente des Kleinhirns, 1882. Consult the last volumes of his work and above all the basic book: Zur Kenntnis des Nervensystems der Crustacen. *Biol. Untersuchungen. N. Folge* Bd. 1890.

(2) V. GEHUCHTEN: La structure des centres nerveux. La moelle épinière et la cervelet. *La Cellule,* Tom. VII, 1892.

1. *Axo-somatic connections by nerve nests rich in fibers* (cerebellum, etc.).

2. *Axo-somatic connections by chalices or baskets poor in fibers* (a, retinal terminations in the amacrine cells of association; b, the chalices of HELD in the nucleus of the trapezoid body; c, central acoustic terminations, etc.).

3. *Axo-somatic connections by terminal nerve tubercles,* with or without pericellular projections *(lateral vestibular glanglion* in fish, reptiles, and birds). The inferior termination of the *bipolar cells* for the rods in the retina, etc.

4. *Individual* or collective *axo-somatic connections* by means of a pericellular nerve bushel, or the encrustation of a crest or brush of neurofibrils (peripheral terminations of the *cochlear and vestibular nerves* in birds and mammals, etc.).

5. *Axo-somatic connections by large nests,* some of which show bulbs or terminal outgrowths *(Endfüssen* of HELD) (Spinal cord and medulla oblongata).

6. *Axo-dendritic connections by climbing fibers* (cerebellum).

7 *Axo-dendritic connections by means of gears* (cerebellar glomeruli, retina of insects and crustaceans, AMMON's Horn, etc.).

8. *Axo-dendritic connections by oblique or cross-like nerve branches* of great length (cerebrum, molecular layer of cerebellum, interpeduncular ganglion, etc.).

9. *Axo-dendritic connections by means of flat and parallel overlapping plexuses* (plexiform layers of the retina, etc.).

10. *Axo-dendritic connections by diffuse arborizations or "Pleiades".*

11. *Connections and terminal arrangements* of some peripheral nerve arborizations (motor plates, corpuscles of GRANDRY-MERKEL, etc.) (1).

Moreover, there are many types of terminations by contact which I omit for the sake of brevity.

I have not been able to verify *dendro-dendritic* and *axo-axonal* connections as described by LEVI in fish (1). I do not deny their possibility; I only declare that in thirty five years of patient work with impregnation methods I have not been convinced of their existence. Nevertheless, I believe that the case probably is due to deceiving appearances to which the best investigators are at times victims.

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(1) In the second part of this paper, I shall discuss helpful arguments taken from neurogenesis, nerve regenerations central pathologic lesions, etc.

I should make a general statement before proceeding. All of the nerve terminations related by contact, as described by me in numerous papers, were presented with absolute evidence and in irrefutable preparations. Terminal arborizations which were pale, mutilated or obscured by imperfections of the staining technique and, with greater reason the abnormal ones (2), have been systematically rejected. In my research I have always folloved a norm which could be called a *statistical* one. I have considered as acceptable and genuine only those arrangements by contact which have been *constant* between both elements of the synapse, especially when one of them appeared strongly impregnated and, even better, when one of them had a slightly different shade. The sections which inspire the most confidence are those in which the neurons appear almost colorless or stained with a shade of rose or red in strong constrast to the black coffee-like color of the nerve arborizations. Such a contrast seems to me to be an unequivocal sign of substantial discontinuity because the sharp interruption of color in the terminal nerve branches implies physico-chemical properties differing from those proper to the intraneuronal structure. The physical contact can attain a marked intimacy but in any case there always exists between both synaptic surfaces an intervening frontier. I shall discuss in another place these and other points or technical criteria and, in particular, the danger of making and studying sections which are too thin, in material such as the nervous tissue where the cells, dendritic projections and axons occupy enormous areas.

NEURONAL MEMBRANE.—The acceptance of a contact between nerve arborizations and neurons presents as a preliminary problem the following: Do the terminal nerve arborizations actually touch the nude protoplasm of the cell or do limiting membranes exist between the two synaptic factors?

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(1) LEVI (G.): *Trattato de Istologia*, Torino, 1927.

(2) Absolute uniformity in the manner of interneuronal connection never exists, even within the same type of cell. The gray substance always offers irregular and even abnormal morphological variations, above all in the cerebellum and the ganglia as I have confirmed repeatedly. To this is added the alterative action of the reagents, especially formol.

I definitely favor this latter opinion, although with the reservation that the limiting films are occasionally so extremely thin that their thickness escapes the resolution power of the strongest apochromatic objectives.

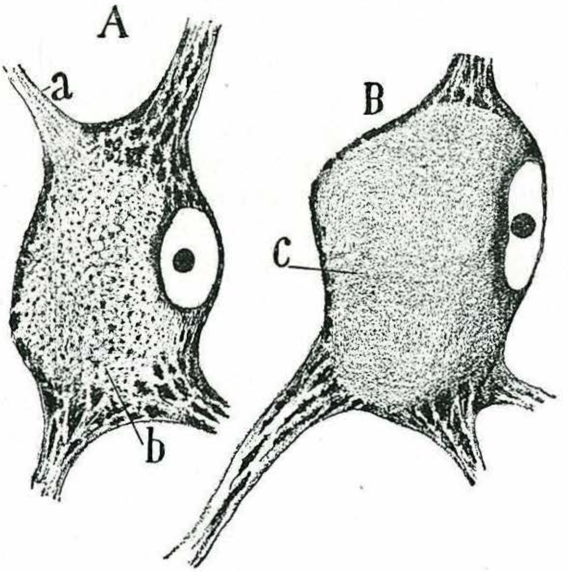

Fig. 1.—Motor cells from the cat spinal cord. Phenomenon of chromatolysis following an injury of the gray matter. Note the tangential nucleus beneath the invisible cell membrane: *b*, *c*, cytoplasm.

I should mention here some of the evidence favoring the existence of such a cuticle:

A) *Direct observation* of a thin cover in the giant cells of the electric lobe in the torpedo.

B) Nissl's *chromatolysis,* showing in the extreme cases a barrier resisting the expulsion of the nucleus and cytoplasm (Nissl and neurofibrillar methods). (Fig. 1.)

C) *Vast swelling of the neuronal somma* in the pathological state and, above all, normal dendrites stained by Ehrlich's methods (marked varicosities) in such a way that this distension causes, at least in the majority of cases, the expulsion of the protoplasmic matter.

D) *Displacement of cytoplasm* and nucleus in certain regions of the nervous system when they are treated with absolute alcohol, followed by the formation of a subcuticular cavity, sometimes of considerable size (ventral acoustic nucleus, etc.).

Pericellular Net.—The pericellular net was briefly mentioned by me in 1897 but was better described by GOLGI (1898), DONAGGIO, MEYER, BETHE, etc. It appears in methylene blue preparations as a fine net having continuity neither with glia nor with neuroglia (GOLGI, DONAGGIO). (Fig. 2.) A minute study of this external structure has persuaded me that it probably consists of a reticulated coagulum provoked by certain fixatives. Today, although I am still in doubt about its preexistence. I feel less skeptical because I have had occasion to observe such a pericellular net not only in GOLGI and EHRLICH preparations, but also in those obtained with a certain special formula of reduced silver nitrate, which incidentally reveals it only in the large cells of short axon in the cerebrum (1). At any rate such a reticulum, preexistent or not, would be independent of the nerve arborizations as GOLGI, DONAGGIO and CAJAL have already noted (2).

My uncertainty concerning the preexistence of GOLGI's pericellular net obliges me, therefore, to have great reserve about the anatomical identification of what LANGLEY designates the *receptive substance.* It is well known that the acceptance of a pericellular material resistant to conductivity has as its aim the explanation of the delay of the propagation of the nerve impulse when it ought to pass from one neuron to another or from an axon to a muscle fiber (chronaxie of LAPIQUE). Without entering into more details, I should note for now that such delay is better reconciled with the neuron theory than with the theory of continuity.

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(1) What increases my doubts is that RAMÓN VINÓS has stained with silver chromate, and photographed analogous nets around the neurons and *within* and *outside of the blood vessels* (unedited work which we are keeping to be published shortly).

(2) CAJAL: Consideraciones críticas sobre la teoría de BETHE, etc. *Trabajos del Lab.*, etc. Tomo II, 1903.

See also: La red superficial de las células nerviosas centrales. *Rev. trim. microgr.*, 1898, tomo III.

If we wish to inform ourselves of the present day conception of the cell membrane, passing from the microscopic to the ultramicroscopic realm, we find that, among the cultivators of colloidal chemistry, all is conjecture and contradiction. It is clear that these scientists have examined the problem from the point of view of the material exchange between the cells and have not considered particularly the case of neuronal synapses.

He who wishes to study these biochemical problems should consult the papers of OBERTON, NATHANSON, TRAUBE, RUBLAND, MÖLLENDORF, etc., which I do not summarize here so as not do depart from the order of my presentation.

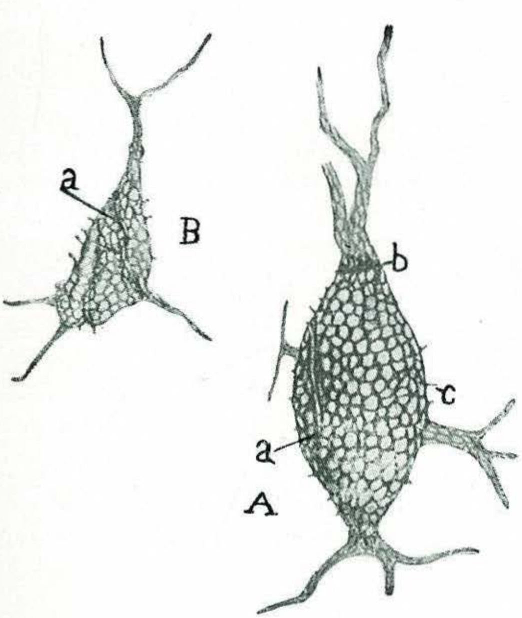

Fig. 2.—Cells of short axon from the cerebral cortex of the cat (EHRLICH's method, slightly modified): *A*, large cell; *B*, small cell; *a*, folds of the pericellular network which could erroneously pass for nerve fibers; *b*, heavily stained polar rings; *c*, spines arising at right angles.

Now let me go on to describe the principal types of synapses, concerning the reality of which I do not harbor any doubt.

CHAPTER III

1.—AXO-SOMATIC CONNECTIONS BY MEANS OF NERVE BASKETS RICH IN FIBERS ("NESTS" OR "BASKETS" OF THE CEREBELLUM, ETC.).

The prototype of this mode of connection, discovered by me in 1888, is found in the cerebellum. The contact is established between the descending nerve branches of the *stellate cells* in the molecular layer and the PURKINJE cell bodies, including the initial unmedullated segment of their axons (fig. 3). This striking relation, demonstrable with the greatest clarity by three methods —GOLGI, COX, and neurofibrillar— was confirmed by KÖLLIKER, VAN GEHUCHTEN, RETZIUS, LUGARO, NAGEOTTE, AZOULAY, ATHIAS, ILLERA, P. RAMÓN, K. SCHAFFER, BIELSCHOWSKY, ROSSI, MARINESCO, WOLFF, LORENTE DE NÓ, ESTABLE and by many other authors.

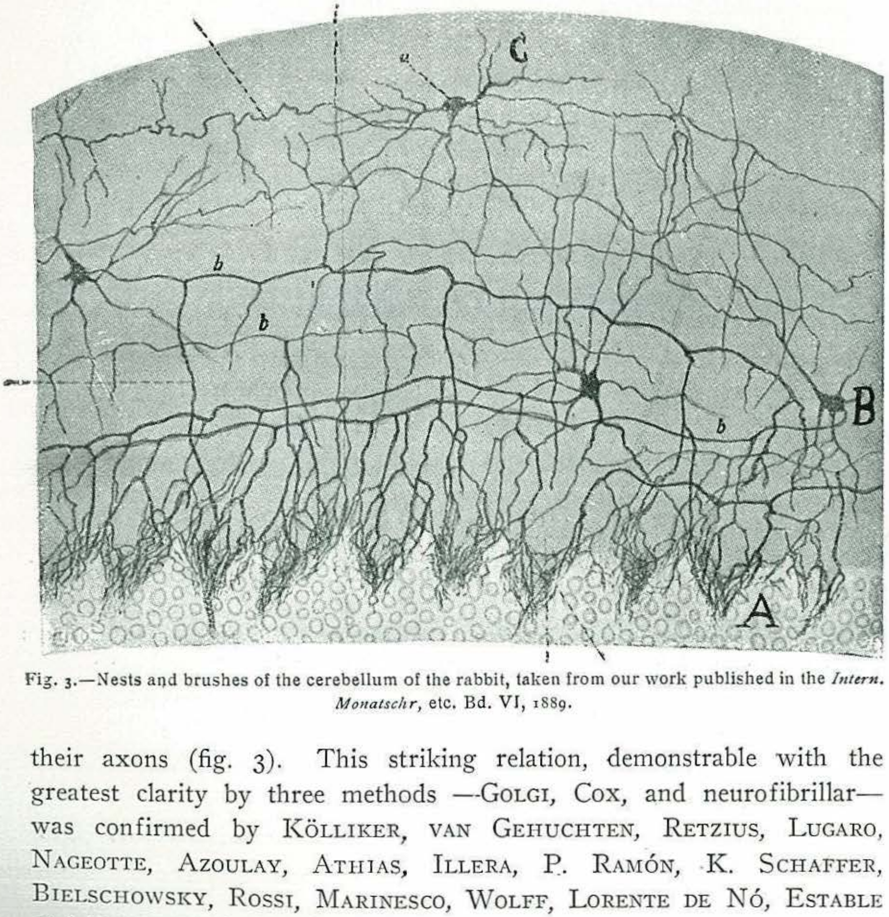

Fig. 3.—Nests and brushes of the cerebellum of the rabbit, taken from our work published in the *Intern. Monatschr*, etc. Bd. VI, 1889.

The discovery of the baskets occurred in the hen where the nests are simple and the terminal nerve branches assume the form of short tassels arranged around the PURKINJE cells. "Never", I said, "in numerous preparations have I been able to find a prolongation of one of the tassels or fringes of these branches coursing through the subjacent *granular layer*. The fibers of which they are formed do not anastomose together, but rather end freely, after becoming markedly enlarged and distended." Later on, in the same year, I found the *nests* in birds and mammals. "In the hen and duck", I wrote, "such tassels or fibrillar skeins are short and cover a small portion of the initial segment of the PURKINJE axon." In birds and mammals the nests are very much longer. "From everything I have said it is inferred" —I added in another paper (1)— "that the fine descending branches of the nerve ramifications from the small cells of the molecular layer have an intimate contact not only with the PURKINJE body but also with the highest and unmedullated portion of DEITER's expansions. Furthermore, does not the hypothesis of transmission of the nerve impulses by contiguity seem to be favored by this peculiar phenomenon of relation, this subordination of the fringe to the cells on the one hand, and on the other to the axons, the direction of which they always follow, enclosing them in a thicket of filaments?" Later on (1889) I published a resumé (2) with numerous details on the *nests* or *descending tassels* as well as on the terminal brushes. This organization, somehow underestimated by the modern authors, has recently been the object of a meticulous analysis by Dr. ESTABLE (3).

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(1) CAJAL: Estructura de los centros nerviosos de las aves. Mayo 1888. *Rev. trimestral de Histología normal y patológica.* Tomo I.

(2) CAJAL: Sobre las fibras nerviosas de la capa molecular del cerebelo. Mayo de 1888. *Rev. trim.*, etc. Barcelona, Tomo I. See also: CAJAL: Sur l'origine & la direction des prolongations nerveuses de la couche moléculaire du cervelet. *Intern. Monatschr.*, & Bd. VI, 1889, and KÖLLIKER: Zur feineren Anatomie des zentralen Nervensystems. *Z. f. wiss. Zool.* Bd. XLIX, H. 4, 1890. Concerning the baskets and brushes of the cerebellum consult also: CAJAL: Las células estrelladas de la capa molecular del cerebelo y algunos hechos contrarios a la función exclusivamente conductriz de las neurofibrillas. *Trabajos,* &. Tomo IV, 1905-1906.

See also: CAJAL and ILLERA: Quelques nouveaux détails sur la structure de l'écorce cérébélleuse. *Travaux,* &. Tomo V. 1907.

CAJAL: Sur les fibres mousseuses et quelques points douteux de la texture de l'écorce cérébélleuse. *Travaux,* &. Tomo XXIV, 1926.

(3) ESTABLE: *Trav. du Lab. de Rech. biol.* Tom. XXI, 1924.

Until 1903 or 1904 all of the research on the nests was carried out with the GOLGI method, which has the advantage not only of staining intensely the arborizations but also of fixing them in an ideal manner, avoiding contraction of the cells in the gray substance. This can be seen in fig. 4, which reproduces a single stellate cell of the molecular layer. The silver neurofibrillar method ordinarily does not stain the cells of short axon, but in 1903 (1), I had the good fortune to observe a happy exception: the cerebellar nests were revealed exceptionally well. The relationship established between the fibers of the nests or baskets (*Endkörben* of KÖLLIKER) is not individual but collective, a circumstance which I have observed in many connections of this kind. By means of its descending branches, which become progressively thicker, each stellate cell establishes contact with many PURKINJE, *cells,* and reciprocally, each one of the latter neurons receives the influence of many *stellate cells* from the molecular layer. In working with young animals or in exceptional though normal cases (since there exists great variety of arrangements), the nest may possess few afferent branches which are frequently found adhering to or encrusted upon the somatic

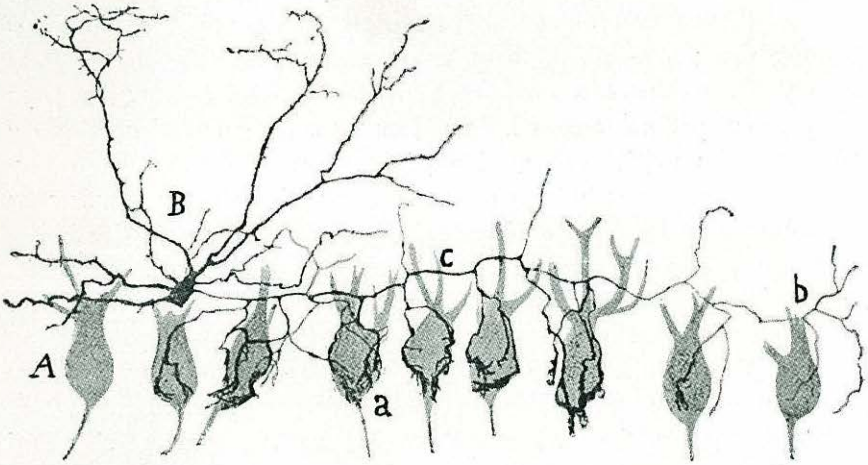

Fig. 4.—A basket cell from the cerebellum of the white rat; *A,* PURKINJE cells; *a,* pericellular ramifications; thick initial portion of the axon; *b,* thin terminal portion with fine descending branches.

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(1) CAJAL: Un sencillo método de impregnación de las neurofibrillas, etc. *Trabajos del Lab. de Investig. biol.* Tom. II, 1903.

surface. However, it frequently happens in the adult animal that such terminal branches are most numerous and some of them, the most superficial ones, seem to be submerged in a special substance, possibly conductive, which extends up to the initial portion of the axon (Golgi method). Recent observations have led me to believe that the majority of these superficial branches are integral elements of the *descending brush* the length and fibrillar richness of which vary a great deal in different vertebrates and even in the same genus of animal. In no case do the descending branches diverge from the brush to be lost in a *diffuse interstitial net* as Golgi and Veratti suppose. *(Opera omnia.* Vol. II, 1903, Milan.) Later on I shall enlarge upon these interpretations of Golgi and his disciples.

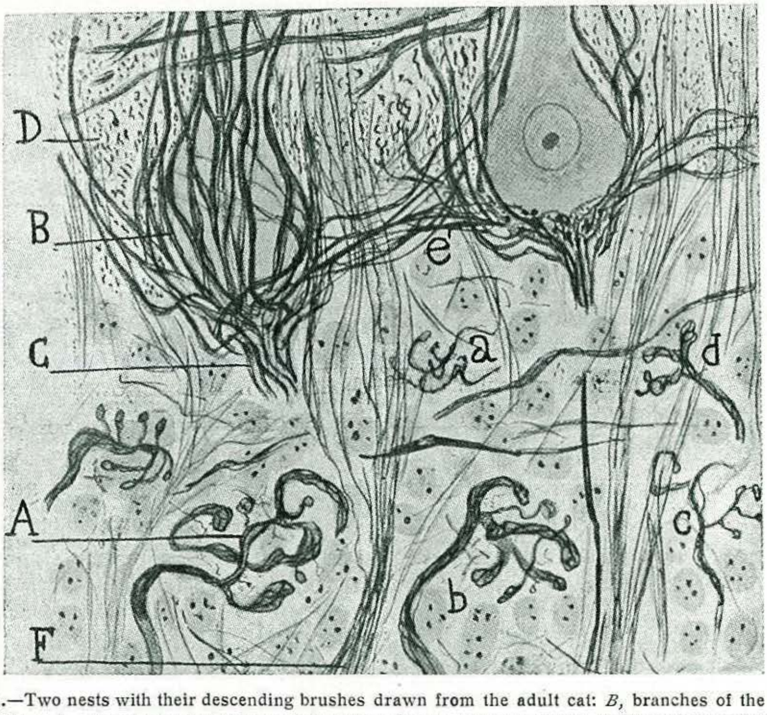

Fig. 5.—Two nests with their descending brushes drawn from the adult cat: *B*, branches of the nest; *C*, brush, *a*, *b*, *c*, terminal arborizations of the mossy fibers; *F*, diverse fibers, for the most part from the granule cell axons which ascend to the molecular layer.

The nests and *bushes,* which I discovered in my first investigation on the cerebellum have been somewhat overlooked during the last few years, despite the fact that they have been observed by many workers and that they are also stained by the neurofibrillar methods. Dr. ESTABLE (1), the Uruguayan neurologist, has made a very minute and critical study of the nests and brushes. He has described all their varieties in the different species of vertebrates and has also pointed out a curious fact, namely, that when an occasional PURKINJE axon emanates from a dendrite, a great many of the branches which generate the nests diverge to arrange themselves in a brush around the initial segment of the axon.

In the higher mammals and above all in man, the *nests* become very complicated. They form tangential or arc-like fibrillar bundles (SCHAFFER's *hammocks)* which coordinate several neighboring E*ndkörben*. Although infrequent, such arrangements are found in birds and rodents as ILLERA and I have demonstrated (1907) (our *associate nests)*.

At any rate the nests were confirmed some time ago by numerous authors already mentioned. They were also observed by AUERBACH, BIELSCHOWSKY, WOLFF, HELD, OUDENAL, etc., although with reservation and caution concerning their significance. The intensity of their color permits one to photograph them easily as I did some time ago (2) and as Dr. JAKOB (3) has done more recently. BIELSCHOWSKY (4) also has recently published a good reproduction of them on the basis of his method of impregnation.

The sections parallel to the cerebellar lamellae show that *in no case do the neurofibrils of the nests penetrate into the protoplasm,* and consequently they are not continuous with the somatic structure. Neither is it possible to observe *any anastomosis among the branches of the nests,* although there are very intimate longitudinal or oblique contacts.

In another section I shall discuss the errors into which the reticularists have fallen. As we would expect, the fundamental fact of transmission of the nerve impulse by contact was untenable to them. Let me formulate here only a general statement. The anti-neuronists began almost all of their studies in man where the nests reach great complexity and exhibit shafts which are not always parallel to the neurons, making it difficult to cut sections with favorable orientation for their analysis. If, instead of this, they would have started their investigations in birds and rodents (mouse and rabbit), passing afterwards to the carnivores (cat and dog), and undertaking finally the problem of the human cerebellum, they would have avoided many doubts, confusions and contradictions. I should add that in man the PURKINJE cells shrink very much under the action of the fixatives and are so far away from one another that it is almost impossible to see the totality of the arborizations of a stellate cell, even with the GOLGI method. Because of these adverse conditions, some authors were unable to identify in man the descending brush which, although it is not absolutely constant, can be recognized in many nests if thick sections are studied. Despite all this, BIELSCHOWSKY has recently described and drawn them in the normal and pathological human cerebellum (1929), as have ESTABLE and the older authors who employed the GOLGI method.

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(1) ESTABLE: Notes sur la structure comparative de l'écorce cérébélleuse. *Travaux du Lab.*, &. Tom. XXI, 1924.

(2) CAJAL: La microfotografía estereoscópica y biplanar del tejido nervioso. *Trabajos,* etc. Tom. XVI, 1918.

(3) JAKOB: Das Zentralnervensystem. Das Kleinhirn. *Handb. d. mikros. Anat. d. Menschen* v. Möllendorff 4, Bd. I. Teil. fig. 142 (microphotographic collaboration by my disciple R. SOMOZA), 1929.

(4) BIELSCHOWSKY: See his articles in the *Hanbd. der mikros. Anat.* v. Möllendorff, 4, Bd. 1928, pgs. 107 and following. As we shall see later, the figures of the Berlin scholar do not differ essentially from mine.

OBJECTIONS TO MY INTERPRETATIONS OF THE BASKETS AND BRUSHES.

A. **Objections of Golgi and his School.**—Despite the exceptional clarity of the disposition of the terminal nests, and notwithstanding the fact that they stain beautifully with the four methods (GOLGI, GOLGI-COX, reduced silver nitrate —almost all of its formulas— and BIELSCHOWSKY), there have arisen doubts and even denials to which we should devote some critical comments.

Some years ago, GOLGI arose in the face of this almost unanimous acceptance by the scientists of the early days. As I have mentioned in passing, he affirmed that the baskets and brushes of the cerebellum, which he did not see until 1902 or 1903, terminate in a diffuse interstitial net in the granular layer, extending even as far as the white substance. I should mention that all the recent investigations with the neurofibrillar methods favor the contrary opinion (ILLERA, ESTABLE, JAKOB, CAJAL, BIELSCHOWSKY, WILDE and CLARK, etc.). Incidentally, in his first paper (1) where he drew the nests or baskets, GOLGI confirmed in general my descriptions of 1888 and 1889, but maintained that the baskets are the result of *incomplete impregnations*, and that good preparations of them are obtained with a great deal of difficulty. He seems to deduce from this that his first impregnations of the cerebellum, in which the baskets and all my discoveries in this organ (baskets, mossy fibers, parallel fibers, climbing fibers, etc.) are absent, would have been the richest and most excellent that could be obtained! What an eloquent testimony to the state of passion a great mind can reach when it is dominated by its theoretical prejudices and by the delusion of its own infallibility!

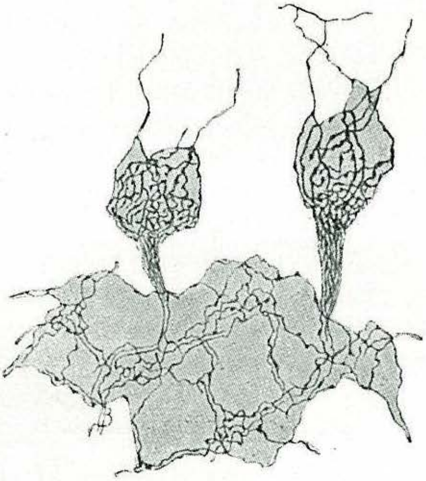

Fig. 6.—Baskets and brushes of the cerebellum which Golgi confirmed in 1906. Copy taken from the Nobel Prize lecture.

In a more modern discourse on the neuron, read on the occasion of the Nobel Prize (2), GOLGI published fifteen years later a figure in which he not only reproduces the *baskets* but also the *descending brushes* (fig. 6). This picture is the most eloquent refutation of his theoretical conceptions because he demonstrates in the baskets an infinite number of fibers which later, when they descend to the granular layer, are reduced to approximately two. (See GOLGI's fig. 2 taken from this speech.) The scholar of Pavia forgot that the axon of the PURKINJE cell invariably emerges from the tip of the brush. At times the *climbing fiber* is associated with it, to say nothing of the axons of the granule cells and the recurrent collaterals of the PURKINJE axon which are in the vicinity of the brush.

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(1) See GOLGI: *Opera omnia*, italian edition. Tom. II, 1903, Torino.

(2) GOLGI: La doctrine du neurone. Nobel price discourse. Stockholm, 1906.

Incidentally, almost all of the authors who have used the GOLGI and neurofibrillar techniques have overlooked a fact which, although infrequent, has theoretical value. According to the observations made some time ago by D. GARCÍA and myself (1) in the rabbit cerebellum, and later by ILLERA and me (2) in some parts of the normal cerebellum, the descending branches of the baskets are prolongated so that they reach the middle or the external third of the *granular layer*, where they generate certain nests destined for some stellate cells with large dendrites. In modern times the patient American histologist ESTABLE (3) has confirmed them.

Here is still another connection by contact. Unfortunately, we do not know the axonal termination of this peculiar type of stellate cell.

B. **Objection of Bielschowsky based on his neurofibrillar preparations.**—The recent opinions of BIELSCHOWSKY concerning the organization of the nests are so close to mine that with a little bit of good will we could attain perfect accord (4). It is known that his conception of the structure of the gray substance is much farther away from the old hypothesis of APATHY, BETHE, NISSL, WOLFF (5) and HELD than it is from my actual point of view.

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(1) CAJAL and GARCÍA: Las lesiones del retículo de las células nerviosas en la rabia. *Trabajos del Lab. de invest. biol.* Tom. III, 1904.

(2) CAJAL and ILLERA: Quelques nouveaux détails sur la structure de l'écorce cérébélleuse. *Trav. du Lab.*, & V, 1907.

(3) ESTABLE: as above. See also CAJAL: Estudios sobre la Degeneración y la Regeneración. Tom. II, 1914. Fig. 255 (there is an English translation by Dr. RAOUL MAY: Degeneration & Regeneration of the Nervous System. Oxford Univ. Press, 1928). And finally, my Histologie du système nerveux, &. Tom. II, fig. 37.

(4) The BIELSCHOWSKY technique, excellent from other aspects, stains the nests very weakly. Nevertheless, by using it skillfully all of my observations are confirmed

(5) The pale, penetrating fibers of the baskets, described and drawn by BIELSCHOWSKY and WOLFF in 1904, surely represent some tenuous, deep fibrils of

When this worker affirms that the baskets or nests contain, besides the free branches, fibers which are foreign to the nerve projections of the *stellate cells* of the molecular layer, he coincides with my ideas, some of them very old, others more recent. It would not be opportune to reproduce here very well known opinions which have been explained by myself, RETZIUS, ILLERA, LUGARO and many others. I should limit myself to present here an outline of this occasional participation by foreign fibers. The accompanying schematic figure (fig. 7, *a* and *b)*, in which I summarize details taken from my old drawings, shows that

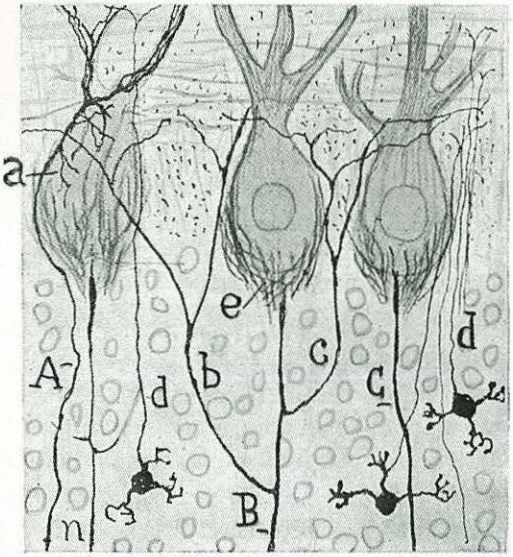

Fig. 7.—Schema to show all the fibers which, as they proceed transversely or tangentially, may accidently mingle with the baskets: *a*, small retrograde branches of the climbing fibers; *d*, axons of the granule cells, etc.

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the nest adhering to the PURKINJE membrane, or perhaps a faintly stained portion of the intrasomatic reticulum. Certainly microphotographs, 19, 20 and 21 in the work of the mentioned scholars fully verify the free termination of the fibrils *from* the baskets. Photography does not bother about partisan prejudices nor suffer from subjective deformations. See the already cited *Memoria* of these investigators: Zur Histologie der Kleinhirnrinde. *Journ. f. Psychol. u. Neurol.* & Bd. IV, 1904-1905. In a recent publication, BIELSCHOWSKY refrains from mentioning the penetrating fibrils.

now and then the climbing fiber and some of its recurrent small branches, the axons of the granule cells, and even the ascending collaterals of the PURKINJE axons intermingle with or border upon the nest (1).

Because this problem has been repeatedly discussed by me in the light of excellent neurofibrillar preparations (very easy to obtain, incidentally, with the BIELSCHOWSKY method as well as the various formulas of silver nitrate; c. f. for example, fig. 5, B) I believe it is superfluous to dwell upon the reticular interpretation of the nests, formerly defended by BETHE, BIELSCHOWSKY and WOLFF, HELD, LACHE, etc.

To complete this critical exposition of the baskets I should note that this organization, more or less simplified, is also observed in reptiles, amphibians and the teleosts. There is, consequently, a structural component common to all the vertebrates which attains its highest development in all those which possess in a high degree the faculty of automatic equilibration, such as the birds and man.

2.—AXO-SOMATIC CONNECTIONS BY MEANS OF BASKETS OR NESTS POOR IN AXONAL BRANCHES.

As a type of this variety of contact having few fibers I should consider what I demonstrated some time ago at the level of the internal granular layer (2) in the avian retina, findings confirmed by DOGIEL (3).

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(1) CAJAL: A detailed analysis of all the mistakes made concerning the structure of the nests and the connections of their fibers will be found in my paper: Sur les fibres mousseuses & quelques points douteux de l'écorce cérébélleuse. *Trav.*, &. Tom. XXIV, 1926.

To verify the participation of the climbing fibers, examine figures 21, 46, 48 and 57 in volume II of the French edition of my complete work. Consult also RETZIUS, *Biolog. Untersuchungen*, N. F. 1890, were he makes note of this peculiarity, which is always exceptional in adult animals and which represents a vestige of the embryonic phase of the climbing fibers.

(2) CAJAL: Sobre unos corpúsculos especiales de la retina de las aves. *Actas de la Soc. esp. de Hist. nat.*, julio 1895.

See also: *Journ. de l'Anat. E. de la Physiol.*, núm. 5, 1896.

(3) DOGIEL: Ein besonderer Typus von Nervenzellen in der mittleren gangliösen Schicht der Vogel-Retina. *Anat. Anzeiger.* Nr. 23, 1895.

Consult also DOGIEL's other works published in the *Anat. Anzeiger* and in the *Arch. f. mikrosk.* Anat. 1888-1894.

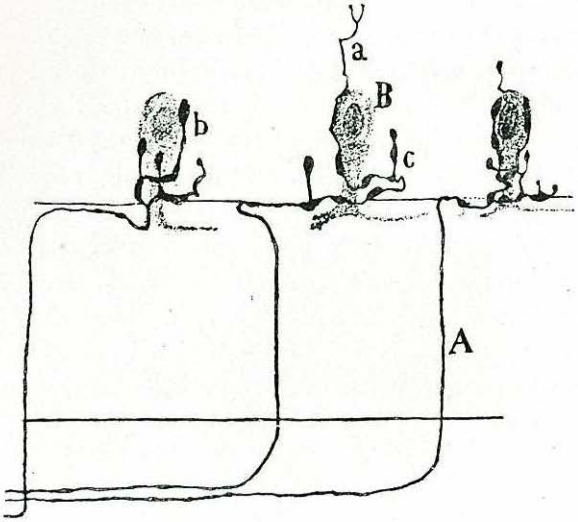

Fig. 8.—Nests formed by the centrifugal fibers of the retina which surround certain types of cells called associational amacrine cells: *A*, centrifugal fibers; *B*, amacrine cell; *a*, *b*, *c*, varicose terminal nerve branches. Methylene blue. Silver chromate gives similar pictures.

In the lower plane of this zone it is observed that certain pyriform cells of short dendrites course toward the subjacent molecular layer.

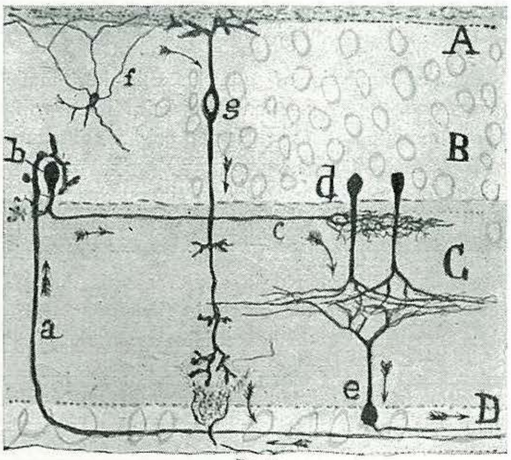

Fig. 9.—Some elements of the retina of birds: *a*, centrifugal fibers; *b*, amacrine cell; *c*, axon of associational neurons.

This axon, as I indicate in figure 8, runs parallel to the zone and terminates by a broad, flat and very complicated arborization.

The most striking detail of these neurons is that the bodies and short, wart-like dendrites are in intimate contact with the terminal arborizations (poor in branches) of certain axons which come from the zone of optic fibers and possibly from the primary visual centers *(centrifugal fibers)*. These strange fibers were also observed by DOGIEL, but in preparations using the EHRLICH method. The deposit of blue, produced now and then in the neuronal articulations when they are stained by ammonium picrate, induced the Russian scholar to take as an origin what in reality represents a true termination. Later on he corrected this error, placing himself in accordance with my opinion.

CHAPTER IV

3.—NESTS OR CHALICES OF HELD IN THE NUCLEUS OF THE TRAPEZOID BODY.

The nests or chalices of HELD were discovered by this author in 1891 (1) but in the following years he somehow overlooked them. Afterwards they were the object of a great deal of research conducted with the GOLGI method as well as with the EHRLICH and neurofibrillar techniques. Among the workers who studied them mention should be made especially of KÖLLIKER (1894), CAJAL (1895), MEYER (1896), LAVILLA (1898), TURNER and HUNTER (1899), VERATTI (1900), VINCENZI (1899), TRICOME-ALLEGRA, MAHAIM, WOLFF, BIELSCHOWSKY and HELD himself, who devoted numerous investigations to them during the advent of the neurofibrillar age. Later I will discuss the opinions of this scholar and the controversies aroused by his reticular theory.

Here I will limit myself to a concise and objective exposition of the irrefutable facts presented by the GOLGI, EHRLICH and neurofibrillar methods.

LAVILLA (2) demonstrated two types of cells in the nucleus of the trapezoid body: those with large chalices of HELD and others, less numerous, provided with very fine cellular nests. These nests may be refractory to the method of BIELSCHOWSKY and even to formulas of reduced silver. I am able to affirm on the basis of neurofibrillar techniques that, both in the nucleus of the trapezoid body and in the other acoustic ganglia, there are special neurons in which these technical procedures do not reveal any kind of nests. The cells in question, triangular or ovoidal are not rare in the cat and rabbit (1).

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(1) HELD: Die zentralen Bahnen der Nervus acusticus bei der Katz. *Arch. f. Anat. und Physiol. Anat. Abteilung,* 1891. I shall speak below about his later works.

(2) LAVILLA, loc. cit. See CAJAL: *Histologie du système nerveux,* etc. Tome I, page 350.

The appearance of the chalices varies greatly depending on whether plasmatic or neurofibrillar methods are used in their demonstration.

With the plasmatic methods (GOLGI and methylene blue procedures, the latter the SEMI-MEYER technique) the afferent fibers of the nucleus of the trapezoid body appear heavy and as they contact the cells they spread out like a species of flower with broad petals notched with complicated indentations. These branches contact intimately all or nearly all of the cell surface and sometimes they send out divergent projections that lose themselves in the thickness of the ganglion. Figure 10, taken from one of my older papers, will give a clear idea of the organization of the chalices in good silver chromate sections. If we use newborn cats, rabbits or mice, instead of animals a few weeks old, the nest assumes the appearance of a solid cone from which many divergent fibrils emanate. This arrangement, first recognized by me in 1895 and confirmed by TRICOME-ALLEGRA in 1904, should be considered as a passing phase of embryological significance.

In adult animals the nest becomes complicated and the provisional or embryonic branches disappear as I (2), LAVILLA (3) and SEMI-MEYER (4) have demonstrated. If methylene blue is used it is observed that the clear interfibrillar spaces of the chalices shrink and at times it seems that the branches themselves anastomose. One receives the impression that the chalice is something external to the cell although very intimately applied to it (CAJAL, LAVILLA, etc.).

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(1) The true thoughts of BIELSCHOWSKY are reviewed in the following words which I take from the treatise of LEWANDOWSKY: "Aus allendem ergibt sich, dass wir auch heute die Neuronenlehre gelten lassen können, wenn wir sie unter Beseitigung der verschidenen Einheitsdogmen als einfache Zellenlehere auffassen".

(2) CAJAL: Algunos apuntes para el estudio del bulbo raquídeo, etc. Madrid, 1895.

See also: El azul de metileno en los centros nerviosos. *Rev. Trim.* Tom. I, 1896.

(3) LAVILLA: Algunos detalles concernientes a la oliva superior y focos accesorios. *Rev. trimestral micrográfica.* Tom. III, 1895.

(4) SEMI-MEYER: Über eine Verbindungsweise der Neuronen. *Arch. f. mikrosk. Anat.* Bd. XLVII, 1896.

If instead of using plasmatic methods we employ neurofibrillar techniques, as proposed first by DONNAGGIO (1) and later by me (1903), the appearance changes. The broad branches, indented like the leaves of a thistle, contract considerably showing a smooth fascicle of fine neurofibrils which seems to make up the skeleton of the chalice. This organization, eventually confirmed by HELD, DONAGGIO, COLLIN, BIELSCHOWSKY, etc., is very easily demonstrated by the latter's method. It is even brought out better by certain formulas of reduced silver nitrate which offer the advantage of exclusively impregnating the neuro-

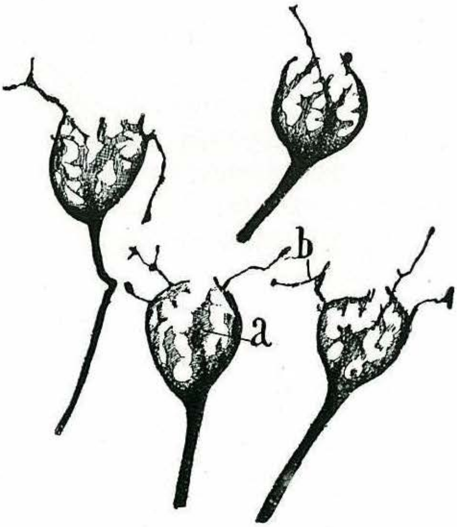

Fig. 10.—Details of the chalices of HELD from the cat of a few days—Golgi method: *a*, principal branches; *b*, thin appendices.

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(1) DONAGGIO: Su speciali apparati fibrillari in elementi cellulari nervosi di alcuni centri del acustico, &. *Riv. sperm. di Freniatria.* Vol. XXIX, f° 1-2, 1903. See his report of 1900: Su alcune particolarità dei centri acustici nei mamiferi (Pavía).

fibrillar skeleton of the chalice in a brownish black color while the protoplasm of the surrounded cell appears colorless or faintly stained in rose or yellow.

Comparison of the pictures obtained with the plasmatic and neurofibrillar methods proves that the principal mass of the chalices is constituted by neuroplasm and not by neurofibrils. The artifacts and precipitate produced at times by silver chromate are accidents that a very careful analysis of a large number of preparations permits us to discard.

CRITICAL OBSERVATIONS OF HELD AND DOUBTS OF BIELSCHOWSKY.

At first HELD was under the influence of the hypothesis of encrustation and later adopted the supposition that the thick, rod-like fibers (1) of the chalices penetrate the cell body. Ultimately he affirmed that a truly substantial continuity exists between the intrasomatic neurofibrils and those of the chalice (2). In this he agrees (although for somewhat different reasons) with GOLGI's school which fearfully noted in these chalices a grave difficulty for the reticular theory.

My former observations demonstrated that the large penetrating fibers of HELD are nothing other than a rodlet of an obscure nature which resides almost constantly in the nucleus of the trapezoid body (3).

Río-HORTEGA (4) published an interesting paper on these rodlets. The affinity of these little organs for the oxide of ammoniacal silver, after preliminary treatment with tannin, has also allowed him to find them in many cells. But today, as we have said above, the histologist of Leipzig has abandoned his early opinion to adopt that of neurofibrillar continuity.

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(1) HELD: Beiträge zur Struktur der Nervenzellen und ihrer Fortsätze. 2. Mitteilung. *Arch. f. Anat. u. Physiol. Anat. Abt.*. 1897.

(2) Zur weiteren Kenntnis der Nervenfüssen und zur Struktur der Sehzellen. *Ges. d. Wiss.*, 1904.

Also: Das Grundnetz der grauen Hirnsubstanz. *Monatschr. f. Psychiatrie &. Neurol.*, LVI, 1927

Also: Die Lehre der Neuronen und vom. Neurencytium. *Fortschr. d. naturwissenschaftl. Forsch.* N. F. Heft 8, 1929.

(3) CAJAL: Un sencillo método, etc. *Trab. Lab. de Invest. biol.* Tomo II, 1903, fig. 13.

(4) Río HORTEGA: Estudios sobre el centrosoma de las células nerviosas, etc. *Trabajos*, etc. Tom. XIV, 1916.

BIELSCHOWSKY also has studied carefully the chalices of HELD and the supposedly penetrating neurofibrils. His opinion, less emphatic than that of HELD, appears in LEWANDOWSKY's (1) book and in MÖLLENDORF's *Handbuch* (2). The impression that reading these works produces is that BIELSCHOWSKY's interpretation is wavering or is in the process of evolution; his figures frequently seem to be confirmations of my ideas (figs. 11 and 12).

In my opinion, the differences in these figures and the indecisions of this scholar are the result of the variety of reactions obtained with the neurofibrillar methods. These diverse images, which I have also observed, can be reduced principally to two:

a) In certain cases the chalice appears strongly impregnated, contrasting intensely with the clear tone of the yellow-reddish or almost colorless soma. There is not the least doubt about its external position as DONAGGIO, using his own technique, noted.

b) At other times the impregnation spreads to the soma and the contrast between the color of the arborizations and that of the neuronal neurofibrils is notably lacking. As a consequence the total picture appears dark and confusing, so that I would say that it belongs to another neuronal type. In summary: its analysis is difficult and produces a great deal of uncertainty. Doubtful pictures of this kind have been drawn by HELD from preparations made with the BIELSCHOWSKY method.

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(1) BIELSCHOWSKY: Allgemeine Histologie und Histopathologie der Nervensystem. *Handb. der Neurologie.* Lewandowsky, 1910.

(2) *Hand. der mikrosk. Anat. des Menschen.* IV. Bd. 1928. Berlin. This book contains two articles by BIELSCHOWSKY alluding to my thesis: *Die Endstrecken der Nervenfasern,* pg. 107, and *Uebersicht über den gegenwärtigen Stand der Neuronenlehre,* &, pg. 119.

See also: *Journal für Psychologie und Neurologie.* Bd. XXI, 1925. Consult the article by BIELSCHOWSKY *(Handb. d. mikrosk. Anat. des Menschen)* in MÖLLENDORF's book of 1928. The surprising thing about this report is that in figure 65 he draws a typical pericellular arborization like the one published in his work of 1925, but in figure 66 the reticulum of the cell is fused and mistaken with a *thick* fiber which does not recall the chalices of HELD in any way. The same contrast is noticed between figure 3 (normal appearance) table V of his *Memoria* in LEWANDOWSKY's book of 1910 and the equivocal ones represented in figure 4 of the mentioned table. (See the figures in this paper, that is numbers 10 and 11.)

The true opinion of BIELSCHOWSKY is summarized in the following words taken from LEVANDOWSKY's treatise (1910): "Aus alledem ergibt sich, dass wir auch heute die Neuronenlehre gelten lassen können, wenn wir sie unter Beseitigung der verschiedenen Einheitsdogmen als einfache Zellenlehre auffassen". In keeping with this restriction, perfectly free chalices are presented in some of his papers (fig. 10).

Concerning the first type of impregnation, I offered sometime ago (1) —and now again present— convincing figures which confirm that the arborization is always around the cell. Similar figures and descriptions have been given by various authors, among them COLLIN, MAHAIM, LAVILLA, WINDLE and CLARK and many others. This very clear image of the superficial chalice composed of heavy, independent branches is the most common and the one which merits the greatest confidence because it is possible to obtain it with many of the silver formulas.

c) HELD and BIELSCHOWSKY showed the termination of the chalice within the cell with the second class of figures because of an infinity of fine and divergent neurofibrils in continuity with the somatic reticulum. These fill me with such great surprise and skepticism that I cannot convince myself of the idea that the shaft which generates such neurofibrils is one of the authentic chalices. The contrast is really remarkable. Let the reader compare the figures from mouse and cat

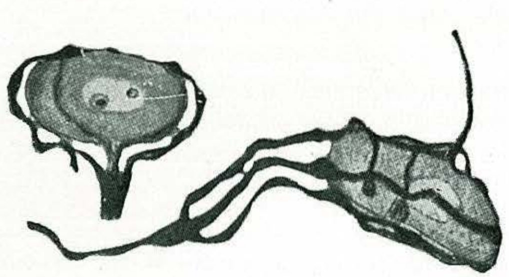

Fig. 11.—Chalices of HELD drawn by BIELSCHOWSKY (first reaction). The terminations appear on the outside of the cell. (In the drawings of BIELSCHOWSKY there is still much more contrast between the cell and the chalice.)

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(1) CAJAL: Un sencillo método, etc. *Trabajos*, etc., 1903. See also: Histologie du système nerveux de l'homme et des vertebres. Tome I, 1909, pág. 108.

reproduced in this paper (figs. 12, 13 and 14) with those of the same kind published by HELD and BIELSCHOWSKY and he will feel the same surprise I did. What then have these scholars been seeing in the dark images corresponding to the second type of impregnation? I offer an example in figure 12, taken from the Berlin neurologist (the drawing on the right).

Several hypotheses are possible to explain this variation in appearances. I shall advance, of course, the one which seems to me more probable. In the dark preparations made by the BIELSCHOWSKY method and in other similar ones obtained with reduced silver nitrate, it is almost always possible (despite some difficulties) to recognize the dark shade of the superimposed chalice, but the lack of clarity hinders me from determining the position of the nerve arborization. I present in figure 15, A, an obscure synapse, the interpretation of which not being difficult, helps us to understand the contradictory character of some of BIELSCHOWSKY's figures.

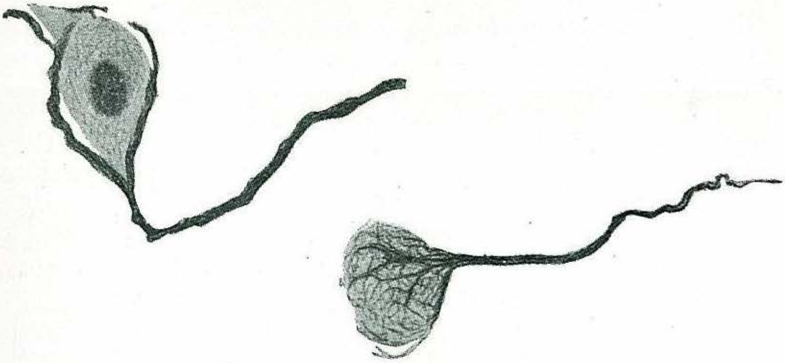

Fig. 12.—Contradictory representations of the chalices given by BIELSCHOWSKY. In the upper appears a normal chalice; in the lower an equivocal figure.

But do all of the images published by HELD and BIELSCHOWSKY positively portray chalices? Figure 12 in the accompanying picture leaves me perplexed. Could it be possible that the fiber taken as a terminal axon is a dendrite? Concerning this point it is important to recall some former observations. Many years ago LAVILLA and I (1) pointed out two facts of some importance: first, not all of the cells of the trapezoid body possess terminal chalices; secondly, besides neurons with heavy chalices there are others surrounded by fine pericellular arborizations.

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(1) LAVILLA: Algunos detalles concernientes a la oliva superior y focos accesorios. *Rev. trim. microgr.* Tomo III, 1899.

If the authority and the interpretative sagacity of HELD and BIELSCHOWSKY were not so great as to sway us, we would be inclined to think that the penetrating fibers of these scholars (fig. 12) represent the origin of neurofibrils which belong to dendrites and which become continuous with the intrasomatic framework of those neurons surrounded by the fine pericellular plexuses. These plexuses are easily stained with silver chromate but almost always escape impregnation with the reduced silver and BIELSCHOWSKY methods (1). On the other hand, in many cells of the nucleus of the trapezoid body and of the ventral acoustic nucleus, the axon and dendrites emerge from a sort of interior arborization. This occurs even in neurons which are surrounded by a chalice. In this respect figure 11 of my *Memoir* (1903) and the axon represented in figure 15, *n* (the nucleus of the trapezoid body of the cat) are very instructive. Furthermore, let me add that BIELSCHOWSKY's supposedly penetrating fiber (fig. 12, right) becomes progressively thinner as it proceeds outwards, thus behaving like a protoplasmic expansion.

Some recent personal observations in the cat and adult mouse.—In order to finish this critical review of HELD's chalices, let me present the results of my recent observations. These were obtained by means of two particulary expressive and effectual silver formulas: that of hydroquinone applied to the sections, and that of successive fixation in formalin and in pyridine which gives excellent and constant results (2)

With these formulas the images appear remarkably vivid and clear. My formulas ordinarily show the chalices almost black and, since the somatic reticulum appears pale, the differences of intensity and shade are so great that any equivocation becomes impossible. Moreover, as I show in figure 13 (method of hydroquinone after the action of silver nitrate, alcohol and pyridine) it is possible to obtain preparations in which the neuronal soma is absolutely colorless and in which no penetrating fibers can be seen.

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(1) BIELSCHOWSKY, as above. The origin of a dendrite by the convergence of relatively thick neurofibrils was already represented by me in my work of 1903 and appears in the axonal cone of origin of some cells (see figure 13 from the adult cat).

(2) See: *Travaux du lab. de Rech, biol.* Tom. XIX, 1921.

Because of the ample clarity of the figures let me terminate this discussion by pointing out some details which confirm former observations. One of them consists of a collateral which abandons the chalice and loses itself among the fascicles of the transversal fibers of the trapezoid body (fig. 13, *C)*. Sometimes at the chalice we observe the arrival of a terminal fiber of uncertain origin (fig. 15, *a)*. Furthermore, we see branches which emerge from the terminal thickening of the heavy axon which generates the chalice; these branches send out a ramifying projection, perhaps to the neighboring neurons (fig. 15, *b)*. And one last detail: a short distance from the cell the pedicle of the afferent axon

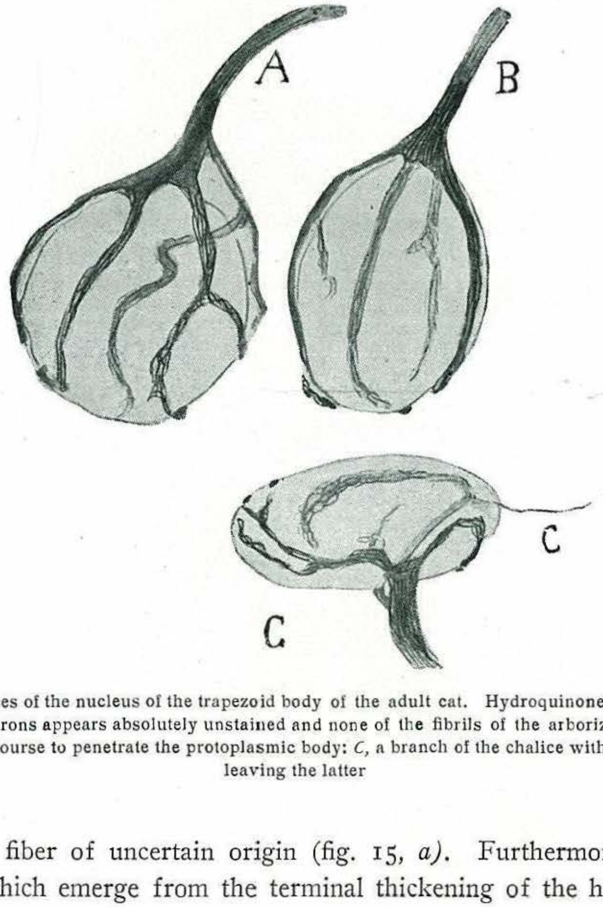

Fig. 13.—Chalices of the nucleus of the trapezoid body of the adult cat. Hydroquinone method. The soma of the neurons appears absolutely unstained and none of the fibrils of the arborizations appears to abandon its course to penetrate the protoplasmic body: *C*, a branch of the chalice with an orientation leaving the latter

suddenly pales before it becomes incorporated with the transversal fibers of the trapezoid body. I believe it to be certain that the medullary sheath begins in this segment.

To be complete we should note further that a contrast exists between the chalices of the mouse and those of the cat. While the latter have great extension and complexity, the former are poor in terminal projections (although these are thick) (fig. 14, *A)*. There are some arborizations which form only a sort of fork emerging from the falciform neurofibrillar formation. It would be interesting to study comparatively the terminal apparatus of various mammals having differences in size and acoustic keenness. Here it happens, as always, the theoretical problems I pose cause me to deviate from the real questions to be resolved.

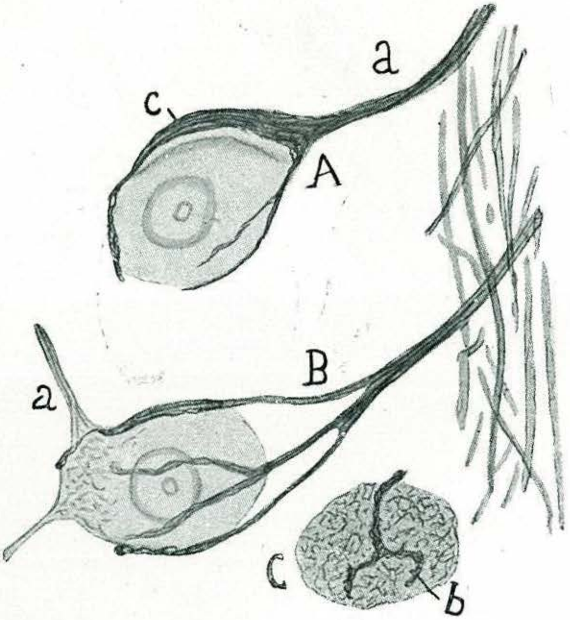

Fig. 14.—Chalices of the nucleus of the trapezoid body of the mouse. (Fixed first in formalin and later in pyridine, stained in block, etc.) In *A* and *B* the independence of the cellular reticulum, barely visible, and of the chalice is self-evident. The tone of the protoplasm is reddish yellow: *C*, section cutting a cell in which the reticulum was stained. It is possible from this that the simultaneous staining produces the illusion of continuity (*b*). Note that the chalice appears vigorously impregnated in a black coffee color.

To summarize: Considering the present state of technology and eliminating all the causes of error, I believe that it is still necessary to maintain the old theory, which was supported (although without insistence) by **Held** himself in his initial work and which states that the chalices are situated external to the cells and represent one of the many variants of perisomatic nests or baskets discovered by modern neurohistology.

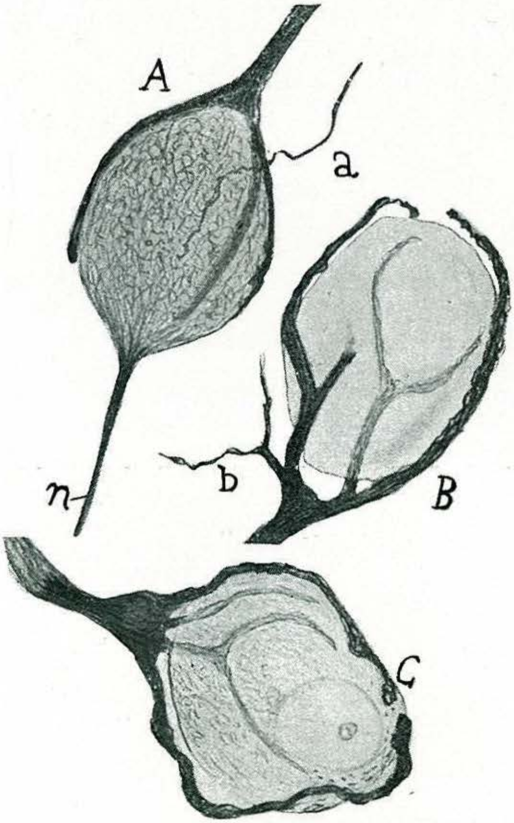

Fig. 15.—Chalices of **Held** from the nucleus of the trapezoid body of the cat: *A*, cell in which the reticulum is stained, although in a red tone; *B*, chalices vigorously impregnated in a deep coffee color; *a*, fiber which arrived at the chalice from a long distance; *b*, a branch which emerges from the origin of the chalice and loses itself in the interstitial plexus; *n*, a well stained axon of a neuron which, without the chalice, might simulate a connection by neurofibrillar continuity. (Method of nitrate-alcohol-pyridine; reduced in hydroquinone.)

4.—AXO-SOMATIC CONNECTIONS IN THE VENTRAL COCHLEAR NUCLEUS.

These connections were described briefly by HELD in 1891 (silver chromate preparations) and they have been studied by many authors whose opinions are far from concordant.

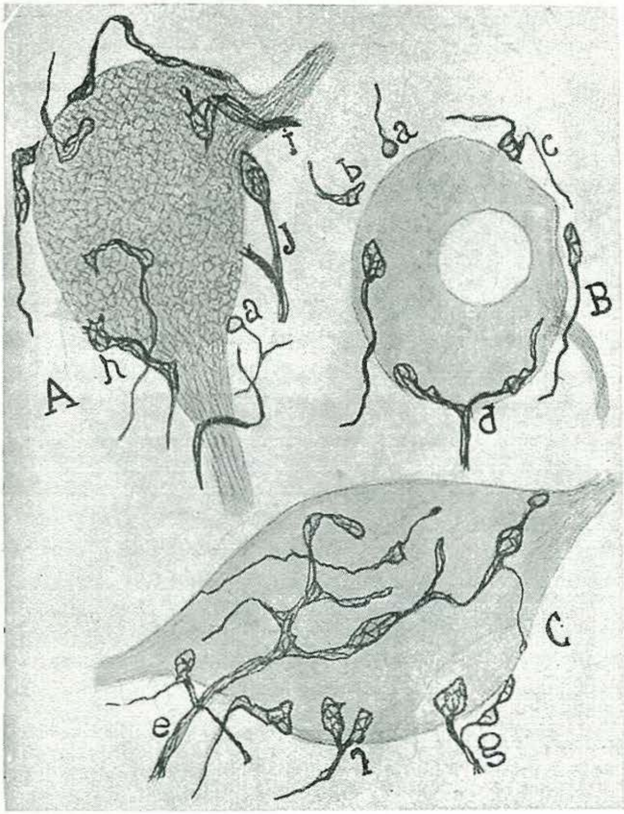

Fig. 16.—Terminal arborizations of the cochlear nerve in the region near the penetration of the nerve (cat): *B* and *C*, cells in which the acoustic arborizations, on account of the reticulum not staining, appears perfectly clear and free; *A*, cell in which the simultaneously impregnation of the protoplasmic reticulum produces the illusion of partial interlacing of the exogenous and endogenous neurofibrils (*i*, *h*); *a*, rings or terminal spheres; *d*, *g*, bifurcated bulbs. (Staining of the sections and reduction in hydroquinone.)

These nests, somewhat similar to HELD's chalices, differ from them in two essential features. First, each neuron of the ventral nucleus, instead of receiving one fiber, receives two or more afferents from the acoustic nerve. Secondly, the terminal ramifications of these axons present irregularly coursing enlargements; frequently, although not always, there are terminal bulbs applied to the neuronal surface (GOLGI and neurofibrillar methods) (fig. 16). As in the nucleus of the trapezoid body, not all of the cells have stainable terminations. There are stellate or triangular neurons around which it is impossible to see any ramifying cochlear axon.

It would not be pertinent to reproduce here my well known studies on the central acoustic arborizations. One desiring detailed information about this question may read my early monographs and above all my book on the nervous system, as well as the evidence I presented in my former controversy with Professor APATHY (1). To avoid repetition I insert here a figure taken from recent material prepared with a hydroquinone formula. A careful inspection of the terminal bulbs and any of HELD's club endings do not permit us to observe any of those penetrating fibrils described by HELD and HOLMGREN. I believe these fibrils are either accidental forms or the result of defective fixation. HOLMGREN has studied the ventral acoustic nucleus of the fox and has drawn, almost exclusively, fine terminal rings (more or less modified by the technique). It is very possible that he has examined different regions than those studied by me. I should not forget, according to my evidence of many years ago, that the closer we approach the origin of the cochlear nerve, the heavier and more complex in organization become the terminal bulbs and arborizations.

CHAPTER V

5.—PERIPHERAL TERMINATIONS OF THE COCHLEAR NERVE IN THE ORGAN OF CORTI. TERMINATIONS OF THE VESTIBULAR NERVE.

A. **Terminations of the cochlear nerve.**—These terminations were discovered by RETZIUS in 1892 (2) in the cochlear nerve of the fetal mouse and were later confirmed by van GEHUCHTEN (1892) (1), GEBERT (2), HELD (3) and myself (4). In recent times they have been studied by KOLMER (5) (reduced silver nitrate), BIELSCHOWSKY and BRÜHL, CASTRO, LORENTE DE NÓ and TELLO, etc.

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(1) Figures analogous to mine were presented by COLLIN (1905) and other authors among whom, as modern writers, we ought to cite WINDLE and CLARK, who have worked with a variant of my method (variant of RANSON) 1928.

(2) RETZIUS: *Biologische Untersuchungen.* N. F. Bd. III, 1928.

Using the GOLGI method, which is still the one offering the most instructive results, RETZIUS proved that the terminal fibers of the cochlear nerve contact the deep pole of the hair cells where they divide into an ascending bouquet which surrounds the cell surface and ends by free terminations. Following the work of the Swedish scholar similar descriptions with few variations were made by all of us in the fleld. utilizing the silver chromate method. Let me reproduce here two figures published by me some time later in which the reader may observe the disposition of the bouquet and its contact with the hair cells. In the accompanying figure (fig. 17, five day old mouse) he will observe that the peripheral expansions of the spiral ganglion in this rodent are arranged in two bundles as RETZIUS had already indicated. One of the fascicles contacts the *internal hair cells* and the other thicker one connects with the external cells. It is never possible to see a continuity between the two components of this connection. Furthermore, the hair cell is almost never stained by the GOLGI method, indicating a different physical or chemical composition.

Later on the use of the neurofibrillar methods complicated the problem of cochlear nerve termination and in a certain sense was a regression. The clarity of the GOLGI preparations was overlooked and the great controversies between the neuronists and reticularists burst forth. Thus BIELSCHOWSKY and BRÜHL (6), who selected the rabbit and two human embryos for their investigation, drew and described as the terminal apparatus of the cochlear nerve, a few, very fine filaments, almost imperceptible, which would penetrate, apparently, into the thickness of the hair cell.

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(1) VAN GEHUCHTEN: *La Cellule*. Tom. VIII, 1892.

(2) GEBERT: *Anat. Anz.* Bd. 8. 1892.

(3) HELD: *Zur Kenntnis der peripheren Gehörleitung. Arch. f. Anat. E. Pysiol. Anat. Abt.* 1897.

(4) CAJAL: Les nouvelles idées sur la structure du système nerveux. París, 1894.

(5) KOLMER: *Anat. Anz.* Bd. 26 y 27, y *Arch. f. mikrosk. Anat.* Bd. 70, 1907.

(6) BIELSCHOWSKY and BRÜHL: Ueber nervöse Endorgane im häut'gen Labyrinth der Säugetiere. *Arch. f. mikroskopische Anat.* Bd. 71, 1908.

My investigations with the neurofibrillar methods, first in birds and later in mammals, confirmed in general my findings with the GOLGI method. As shown in figure 17 (fetal rabbit at term, the stage when CORTI's tunnel begins to form) (1) the peripheral expansions of the cochlear nerve are distributed in two bundles as had already been indicated by RETZIUS and other authors. One of them runs to the hair cells of the *internal lip* (A); the other courses to the more numerous hair cells of the *external lip* (B). The more striking fibers of these fascicles (exclusive of the spiral bundles) contact the epithelium, pass between the supporting fibers and, arriving at the *hair cells,* end in a ramification of short filaments which intimately surround the internal pole of these cells. The number of such terminal branches is variable but never very great. Nearly all the neurofibrillar filaments are localized in the infranuclear region of the protoplasm, in the interior of which

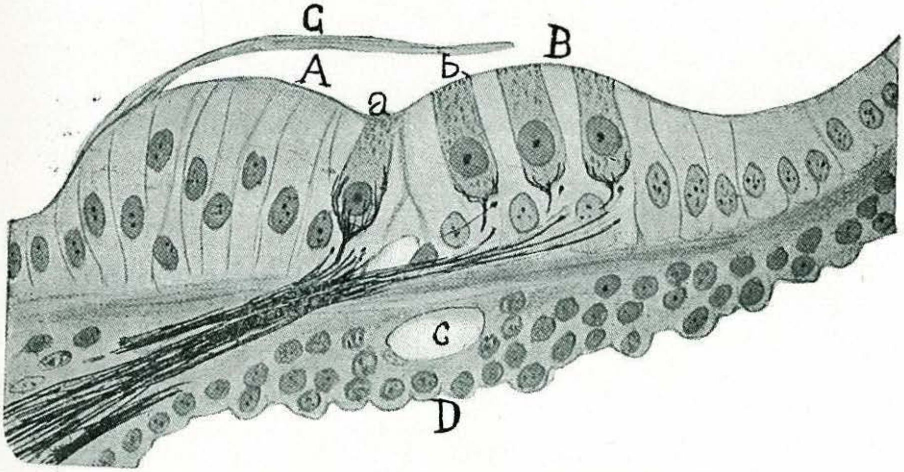

Fig. 17.—Section of the epithelium of the organ of CORTI from the mouse fetus at term: *A*, internal border; *B*, external border; *D*, scala tympani; *a*, inner hair cell; *b*, outer hair cell; *c*, spiral vessel (fixation in pyridine).

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(1) CAJAL: Acción neurotrópica de los epitelios, etc. *Trabajos,* etc. Tom. XVII, 1919.

they are never observed. We are dealing, therefore, with a most intimate contact as is the general rule with all axonal terminations.

Some years previously (1) I also described a similar kind of termination in the cochlea of young birds. As in mammals, the axon arrives at the *Pars inferior* of the organ of CORTI where it spreads out in a thick bundle of neurofibrils which terminate freely around the deep pole of the hair cells in a union so intimate that a free terminal *bouquet* is never seen. Here is a case where one can see well what HELD would call termination by encrustation. In birds we neither detect the intrasomatic penetration of neurofibrils nor the formation of those vases or elongated chalices characteristic of vestibular nerve terminations. In general it seems to me that the terminal *bouquet* is much richer in birds than it is in mammals (2).

The preparations of CASTRO, using his method of decalcification in adult or nearly adult mice, permits us to observe the same organization. In the cochlear as well as in the vestibular nerve, the terminal filaments are always external to the cell and the ramification is invariably poor in perisomatic fibrils. Thus my observations, except for the interpretation of anastomoses, coincide with those well drawn figures described by BIELSCHOWSKY and BRÜHL, according to whom, the neurofibrillar skeleton of the *bouquet* of RETZIUS is reduced to a few fine filaments.

Needless to say, if the independence of the terminations of the cochlear nerve is evident in the adult or late fetus, it will be much more so in the early embryo where the organ of CORTI is still unmodeled and hair cell differentiation is not distinguishable. I show in figure 18, D, the primordial phases of the penetration of the cochlear fibers (mouse fetus, 1 cm.). Note how the ascending fibers (D) arise from the spiral ganglion then, running at random among the undifferentiated epithelial elements, terminate in sharp points. This arrangement has recently been verified by LORENTE DE NÓ (1) and TELLO, who, employing the method of preliminary decalcification in the fetal mouse, have carefully studied the successive stages of cochlear nerve terminations.

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(1) CAJAL: Terminación periférica del nervio acústico de las aves. *Trabajos del Lab. de Invest. biol.* Tom. VI, 1908.

(2) F. DE CASTRO: Technique pour la coloration du système nerveux quand il est pourvu des etuis osseux, pgs. 443 and 444. *Trav. du Lab.* &. Tom. 23, 1925. In this paper, CASTRO is specially interested in the vestibular terminations, but his sections have persuaded us that an analogus organization exists around the hair cells of the organ of CORTI, except that the chalices possess fewer rami. In any case, CASTRO's transversal sections prove that the arborization is exterior.

Lately I have analyzed the cochlear terminations in sections almost parallel to the *tectorial membrane* in the mouse a few days old (figure 19). In agreement with my former investigations the *bouquet* appears meager and external. On the other hand, an internal neurofibrillar structure can be recognized in the hair cell which recalls the drawings of KOLMER. This reticulum seems sparse in the external part of the hair cell but

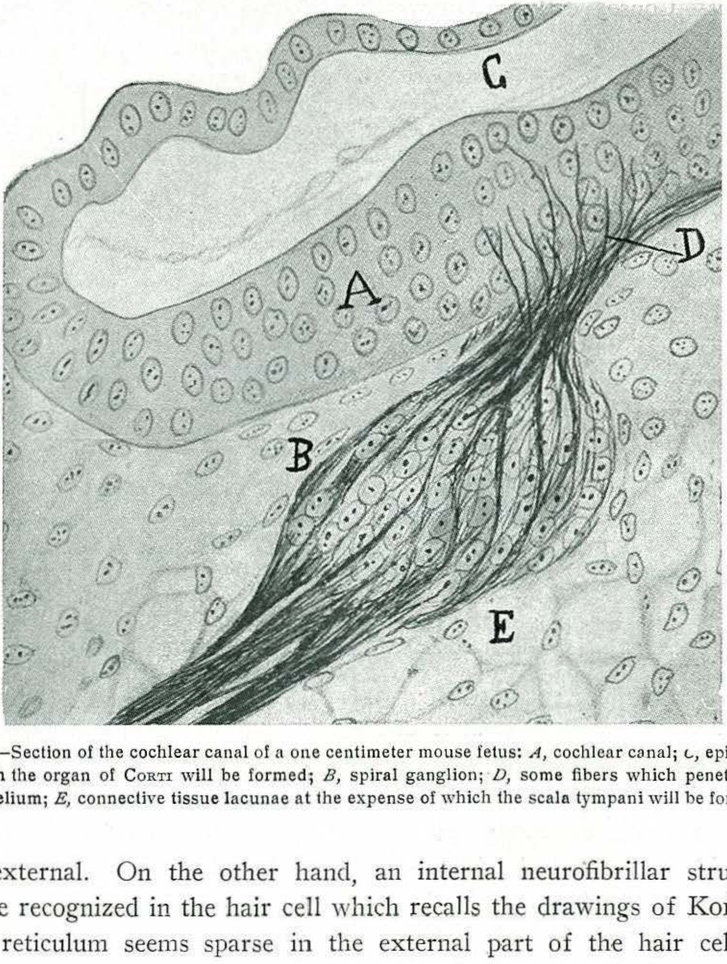

Fig. 18.—Section of the cochlear canal of a one centimeter mouse fetus: *A*, cochlear canal; *C*, epithelium in which the organ of CORTI will be formed; *B*, spiral ganglion; *D*, some fibers which penetrate the epithelium; *E*, connective tissue lacunae at the expense of which the scala tympani will be formed.

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(1) LORENTE DE NÓ: Etudes sur l'Anatomie et la Physiologie du Labyrinth de l'oreille et du VIII nerf. *Travaux*, &. Tom. XXIV, 1926.

complicated and compact in the deep pole *(a)*. The independence between the afferent and intracellular neurofibrils is evident.

In summary: the technical difficulties, interfering with a good analysis of the peripheral organization of the cochlear nerve in the organ of Corti, explain these differences of opinion. Despite this, all good preparations obtained in the fetus and animals, both young and adult (mouse), are evidence in favor of an interneuronal connection by contact. Consequently, I believe that if this study could be made in man and the higher mammals one would find, except for difference in detail, the same facts.

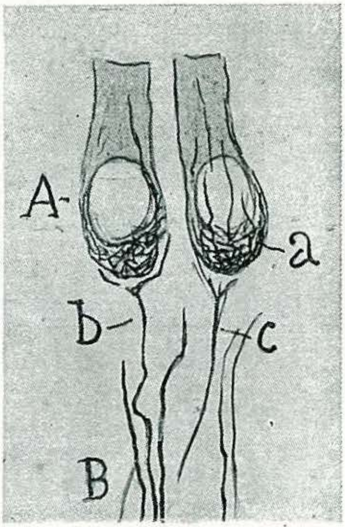

Fig. 19.—Two hair cells of the organ of Corti with their cochlear terminations: *A*, *a*, hair cells; *B*, cochlear fibers; *b*, terminal ramifications. Mouse of a few days (fixation in pyridine).

B. **Peripheral terminations of the vestibular nerve.**—Fortunately well illustrated documents and exact observations abound in this domain. This is understandable if one recalls that these terminations are beautifully and intensely stained with the Golgi and neurofibrillar methods in mammals as well as in birds and reptiles. When these preparations are interpreted objectively and judiciously, the neuron theory finds therein one of its strongest foundations.

The Golgi method, in the hands of such expert investigators as

RETZIUS (1) and LENHOSSÉK (2), offered the first positive data concerning the manner of termination of the peripheral branches of the bipolar cells of Scarpa's ganglion. NIEMAK (1892), KRAUS, GEBERT and others applied EHRLICH's method to this problem. After the neurofibrillar methods had been devised, I made some contributions choosing, preferably, the avian vestibular nerve which is particularly suited to such research (3). Further, we should not overlook the numerous investigations carried out by KOLMER (4), who used my methods, or the important work of BIELSCHOWSKY and BRÜHL already referred to. Although I shall not cite all of the present day workers it is necessary to mention LORENTE DE NÓ (5), CASTRO (6), POLJAK (7) and TELLO (8).

An accurate bibliographical study would reveal that differences of opinion depend both on the methods employed and the degree of development of the animal used. Those who have employed the GOLGI method such as RETZIUS, LENHOSSÉK and myself (9), advocate termination by contact because the afferent fibers always run on the outside of the hair cells of the *cristae* and the *maculae* of the labyrinth. (An exception was Ayers who thought he observed a substantial continuity.) On the contrary, the majority of those who have worked with the Erlich method, such as Niemak, Krause, Gebert and others, maintain that the vestibular fibers spread out, forming a pericellular cup (Kelche) or are connected with a special substance which surrounds the greater part of the *hair cells* (Niemak).

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(1) RETZIUS: Die Endigungweise des Gehörnerven. *Biol. Untersuchungen.* N. F. Bd. III, 1892. The important observations of RETZIUS include bird embryos (chicken, from the eleventh to the seventeenth day of incubation) as well as new born and four day old mice.

(2) V. LENHOSSÉK:Die Nervenendigungen in der Maculae und Cristae acusticae. *Versammlungen der anat. Gesell. zu Göttingen,* 23 Mai, 1893.

(3) CAJAL: Asociación del método del nitrato de plata con el embrionario, etc. *Trab.* etc. Tom. III, 1904. See also: Histologie du système nerveux. Tom. I, pgs. 755 and following.

(4) KOLMER: *Anat. Anz.* Bd. 26 and 27.
Also: *Arch. f. mikrosk. Anat.* Bd. 70. 1907.

(5) LORENTE DE NÓ: As above.

(6) CASTRO: As above.

(7) POLJAK: Ueber die Nervenendigungen in den vestibulären Sinnesendstellen der Säugetiere. *Z. f. Anat., &. Entwicklungsgesch.* Bd. 84, 1927. POLJAK worked with silver chromate in the rat, revealing singularly robust or lamina-like terminal chalices.

(8) F. TELLO: Le reticule des cellules ciliées du Labyrinthe chez la souris et son independance des terminations nerveuses de la VIII paire. *Trav. du Lab. &.* Tom. XXVII, 1931.

(9) Besides V. LENHOSSÉK, who studied fairly advanced stages in the development of the maculde, RETZIUS and I used silver chromate before the differentiation of the hair cells intervened. For this reason we did not see the horizontal plexuses pointed out by the Hungarian scholar. Later on I fully confirmed them, as well as did CASTRO in the work mentioned above.

Finally, the authors who attack the problem using my old neurofibrillar method (formula 1) as Kolmer, London and London, Pesker and others,

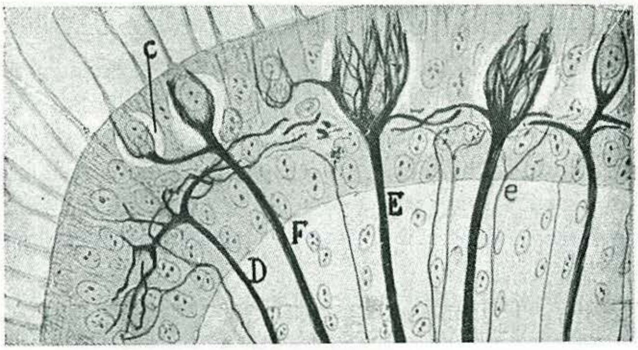

Fig. 20.—Chaliced terminations of the vestibular nerve of birds in the epithelium of the crista ampullaris: *E*, giant fiber which forms the nests for three hair cells; *D*, *e*, fine fibers distributed in a horizontal plexus beneath these cells.

are inclined to postulate a continuity between the reticulum of the *hair cells* and the *external chalice* (1), although some of them also admit free ending fibers. Bielschowsky and Brühl, without any hesitation, maintain the theory of continuity, though the scarcity of terminal neurofibrils observed and their extreme thinness suggests that the method used by them does not reveal the entire arborization (2).

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(1) See figures 21 and 23 of Kolmer's article already cited.

(2) It is just to recognize that Bielschowsky and Brühl discovered a new structural factor within the hair cells and below the nucleus — a delicate neurofibrillar ring which has been confirmed recently by Tello in the adult mouse. We also owe to these scholars the first tentative success in using the Bielschowsky method with decalcified material.

Perhaps it will be asked: how is it that the results obtained by the neurofibrillar methods differ so much, even to the point where I detect unquestionable discontinuity in young birds while others resolutely defend continuity?

The reasons for this are diverse and simple: it all depends upon the neurofibrillar formulas employed which produce three different effects very prone to equivocal interpretation. As causes of discrepancy I should also add the age of the fetus, the species of animal used and, above all, the extraordinary intimacy of the contact.

Thus, for example, if we make use of the reduced silver nitrate method (formulas with fixation in ammoniacal alcohol or pyridine) and choose birds or new born mammals, the hair cell appears colorless while the terminal chalice is very vividly stained. This happened to me in the beginning with the chicken embryo and recently with birds a few days old. In such preparations discontinuity is very evident because the internal reticulum of the surrounded cell has no affinity for silver. TELLO, especially, experienced the same results even in the mature, or

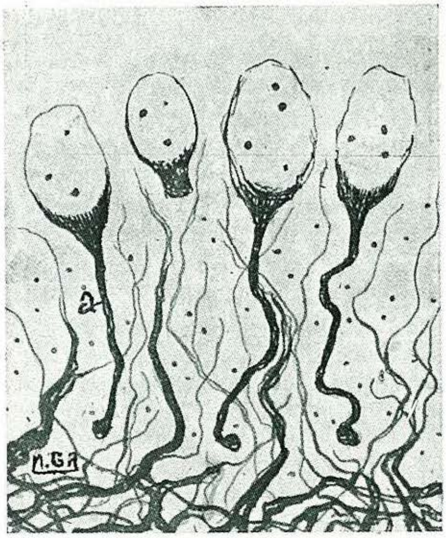

Fig. 21.—Ten millimeter mouse embryo. Note the inferior situation of the hair cell neurofibrils and the existence of a descending pedicle *(a)*. (TELLO).

nearly mature, mouse (fixation with chloral nitric acid, formula of CASTRO) (1).

On the contrary, when the *first formula* of the reduced silver nitrate method is used. following the practice of KOLMER, the internal framework of the hair cells and the neurofibrils of the chalice or the terminal cup sometimes stain simultaneously.

Finally, when the reduced silver nitrate formulas are applied to young embryos (mouse, pyridine fixation) the reticulum proper of the *hair cells* is at times stained with singular beauty in a strong brown or almost black color. On the other hand the fibers of the vestibular nerve are impregnated with a pale rose color and are disposed in the horizontal plexuses discovered by LENHOSSÉK, without the least tendency of entering into relation with the above mentioned internal reticulum. In these admirable preparations no error is possible because the chalices or cups of the hair cells are not yet formed.

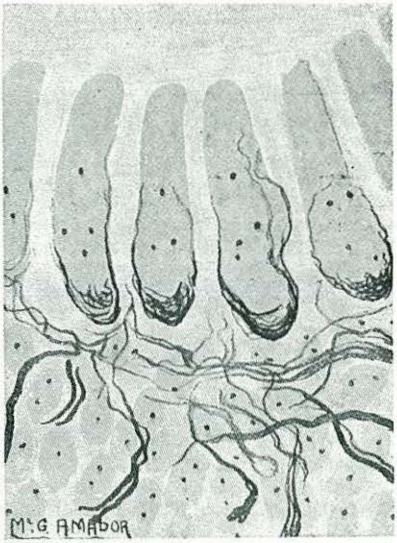

Fig. 22.—Elongation of the hair cells and the appearance of the vestibular plexuses. The pedicle is atrophied. Mouse embryo from 9 to 10 millimeters. (TELLO).

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(1) As above.

This is what TELLO has recently clarified, explaining with great clearness the causes of contradiction among the scholars (1).

As is apparent in figure 21 (10 mm. embryo) the *hair cell* is provided with a dense polar reticulum which is prolonged, in the form of a handle, almost to the basal membrane of the embryonic epithelium. At the same time the vestibular fibers, fine and few in number, lose themselves, without forming a chalice, in the epithelial mass where they end freely and in complete disarray.

Figure 22 shows a stage in which the descending expansion of the hair cell has atrophied and some superficial neurofibrils grow in a radial direction (9-10 mm. embryo). We should note the absence of interlacing between the hair cells and the vestibular nerve fibers which are not yet connected.

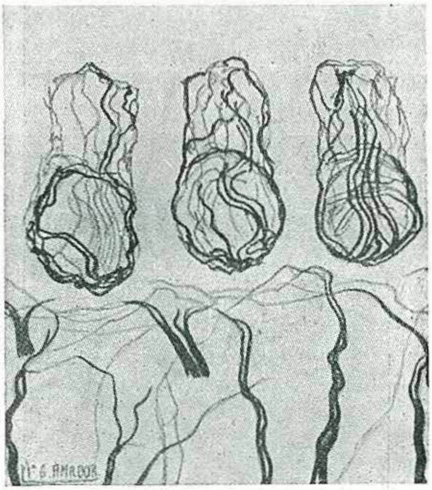

Fig. 23.—17 millimeter mouse embryo. Complete development of the neurofibrillar framework of the hair cell and its independence from the vestibular fibers. (TELLO).

But the stage reproduced in figure 23 (17 mm. embryo) is especially significant, since it shows complete independence of the *hair cell* neurofibrils (extraordinarily apparent) and of the afferent vestibular fibers which have not yet initiated the formation of the terminal cups.

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(1) TELLO: El retículo de las células ciliadas y su relación con las terminaciones nerviosas. *Bol. de la Soc. esp. de Hist. Nat.*, Julio de 1930.

See also his more recent and extensive work: Le reticu'e des celulles ciliées du Labyrinthe chez la souris et son independance des terminaisons nerveuses de la VIII^e paire. *Trav. du Lab.* &. Tom. XXVII, Marx. 1931.

Finally, figure 24, which represents the terminal vestibular apparatus of the adult mouse, is decisive. In the *hair cell* at the left the reticulum appears in a stage of partial regression with full and rich development of the *nerve cup,* while in the cell at the right the cup is totally detached due to protoplasmic retraction. It is very possible that the framework of the hair cell remains more or less altered; at any rate its staining becomes very difficult and unsatisfactory.

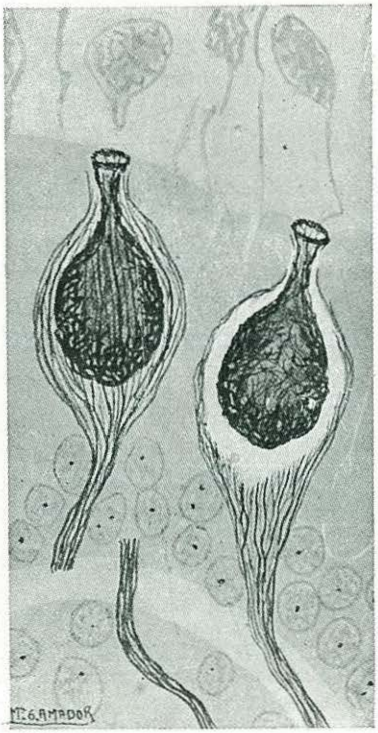

Fig. 24.—Pericellular terminations of the vestibular nerve in the adult mouse. Note in the figure on the right the independence of the endogenous and exogenous reticulum. (Tello).

In summary, from the multiple and apparently contradictory observations carried out by many investigators with different methods of impregnation, the following conclusions are warranted. First, the hair cell has its own independent reticulum and as this cell develops, the reticulum atrophies and almost disappears in certain animals (1). Secondly, the afferent vestibular fiber around this cell ends by means of a rich arborization in the form of a chalice after sending off tangential fibers to the plexus of LENHOSSÉK, a plexus which I have confirmed in birds. Thirdly, there is a period of development in which both neurofibrillar plexuses, *intracellular* and *extracellular,* are stained simultaneously. But an accurate analysis of transversal sections demonstrates the complete independence of the two, as CASTRO and TELLO have shown. Therefore, the termination of the vestibular nerve, far from constituting an insoluble difficulty to the neuron theory, represents, on the contrary, one of its most decisive and brilliant proofs.

CHAPTER VI

6.—AXO-SOMATIC CONNECTIONS BY MEANS OF THICK TERMINAL TUBERCLES WITH OR WITHOUT PERICELLULAR PROJECTIONS. (RETINA.) TUBERCLES OF THE VESTIBULAR NERVE IN THE LATERAL NUCLEUS OF THE MEDULLA IN BIRDS AND FISHES. BULBS OF HELD. "ENDFÜSSEN" OF THE MOTOR NERVES.

A. **Axo-somatic connections in the retina.**—The best example of this simple type of interneuronal relationship is shown with exceptional clarity in the retina of fishes and mammals. As indicated in figure 25 (GOLGI preparation) the *bipolar cells for the rods* (d) have a descending prolongation, similar to an axon, which crosses the *internal plexiform layer* (F) to reach ganglionic layer (G) where it thickens and terminates by means of a tubercle. At times irregular. warty excrescences arise from this tubercle and end upon the external surface of the soma of the ganglion cells. Figure 25 (fish retina) dispenses me from describing the fine details which can be found in my extensive work on the retina (1).

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(1) Understand that to me *disappear* in this case does not signify to cease to exist (that would be hazardous assertion), but to lose completely or almost totally the affinity for colloidal silver. It would be possib'e to cite hundreds of examples of this antipathy for the colloidal silver as the developement of the neurons takes place.

It is important to note that the above mentioned *bipolar cells for the rods* (which receive the terminal spheres of the descending basilar fibers at their external poles) are stained only by the GOLGI method. The EHRLICH procedure very seldom shows them and then not in a perfect manner, while the neurofibrillar methods are never successful. The severe critics of the GOLGI method ought to meditate upon this fact in order to get the idea that many neurons lack neurofibrils or have no affinity for colloidal silver (2). There are numerous examples of this

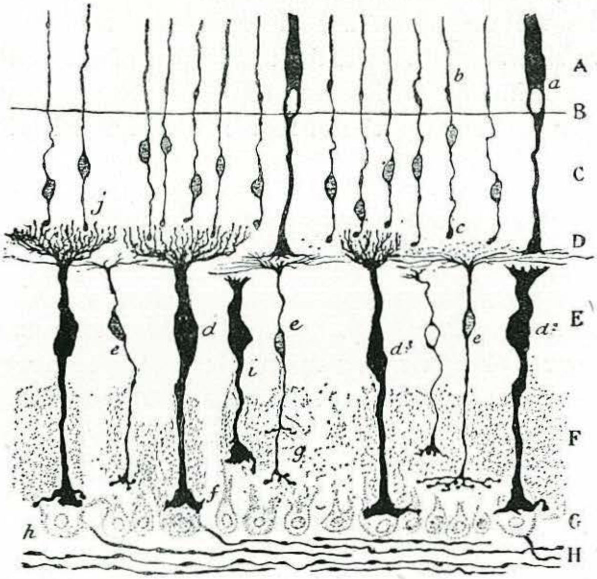

Fig. 25.—Transverse section of the retina of Cyprinus carpio: *A*, layer of cones and rods; *B*, external limiting membrane; *C*, external granular layer; *E*, internal reticular layer; *G*, layer of ganglion cells; *H*, layer of optic nerve fibers; *a*, cones; *b*, rod; *d*, giant bipolar cell; *e*, thin bipolar cell.

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(1) See my book: "La rétine des vertebrés", *La Cellule*, 1892, and the Tom. II of the "Histologie du système nerveux, &.", Paris 1911, Chapitre XV et XVI.

Some time ago I devoted a special study to the retina of the teleost where the giant cells for the rods appear (*Act. de la Soc. esp. de Hist. natural.* Tom. II, Junio de 1892.

(2) By a fortunate exception there are certain central and peripheral nerve terminations very rich in neurofibrils (cerebellar nests, central and peripheral

in all parts of the nervous system. After all, the protoplasmic mass of the dendrites and nerve cell bodies is important.

B. **Tubercles of the vestibular nerve in the lateral nucleus of the medulla in birds, reptiles and fishes** (1).—These formations are well known for they have been described in a journal of great

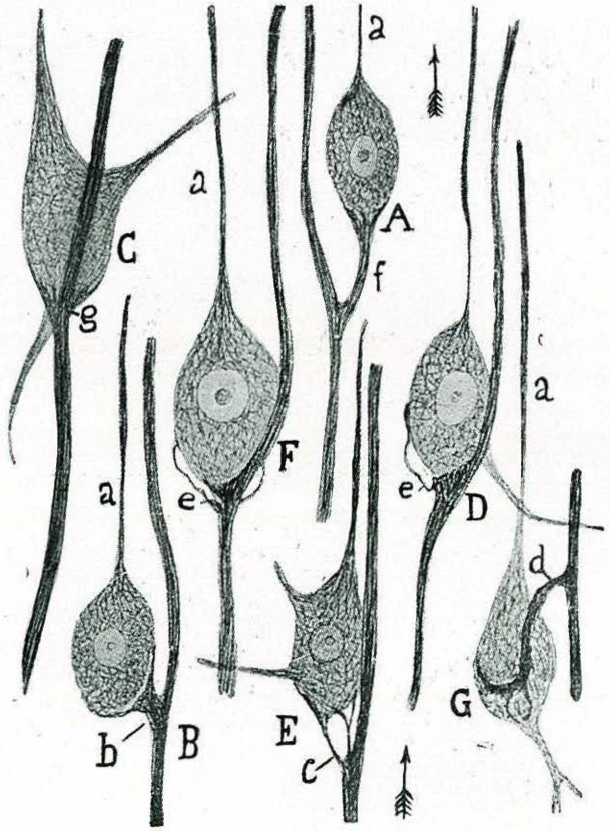

Fig. 26.—Cells of the tangential nucleus of the bird (Kite of a few days): *A, B, C, D,* cell receiving a terminal excrescence; *a*, cone of these neurons; *b, c, d, e, f, g,* diverse dispositions of the connecting branch emitted by the fibers of the vestibular nerve.

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acoustic terminations, pericellular plexuses of the spinal cord, horizontal cells of retina, etc.). On the other hand the great majority of the nuclei in the thalamus, striate body and midbrain, etc., lack stainable fibrils.

(1) CAJAL: Sobre un ganglio especial del nervio vestibular en las aves y en los peces. *Trabajos del Lab. de Invest. biol.* Tom. VI, 1908.

circulation and reputation (1), so I shall limit myself here to a brief exposition.

My so-called *tangential nucleus* is located in the thickness of the vestibular nerve immediately after its entrance into the medulla. It consists of large, ovoid cells with one or more divergent dendrites

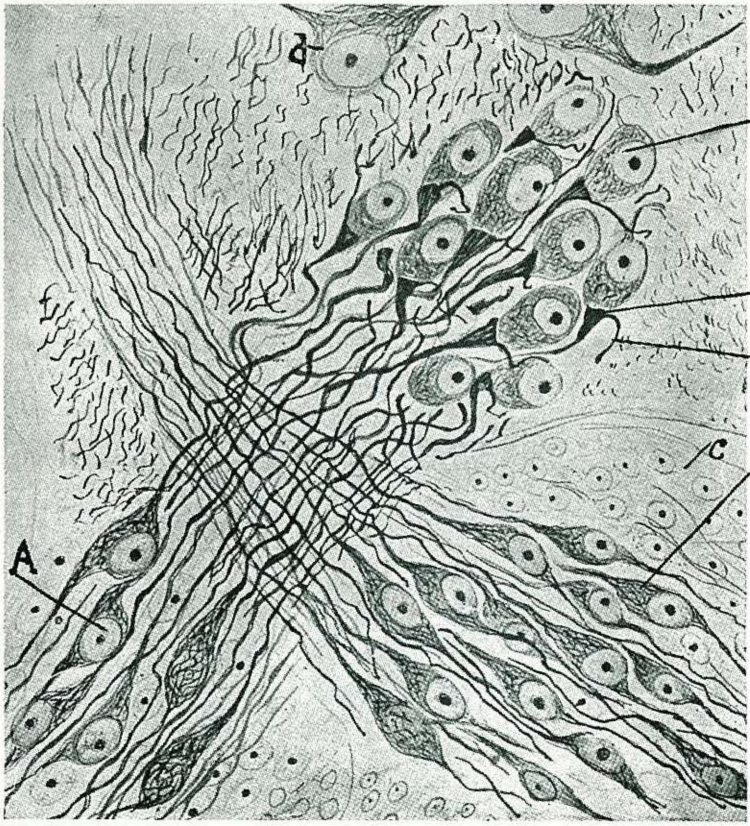

Fig. 27.—Lateral portion of a bulbar section of the trout. (embryo of 14] days): *A*, external lobule of Scarpa's ganglion giving origin to the huge fibers of the tangential nucleus: *B*, ventral lobule; *C*, tangential nucleus; *a*, connecting excrescence; *b*, descending branch of the vestibular nerve; *c*, inmature cells of Scarpa's ganglion.

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(1) CAJAL: Les ganglions terminaux du nerf acoustique des oiseaux. *Journal für Psychol. und Neurologie*. Bd. XIII, 1908.

(fig. 26). But what is interesting here is to point out the peculiar connections of these cells with the nerve. The vestibular nerve is divided, as it is in mammals, into an ascending branch directed toward the cerebellum, and a descending, or better, a horizontal one which is lost in a ganglion probably homologous to that of Deiter's. Now before its bifurcation, the shaft of the axon sends off a thick and heavy collateral which, expanding into a triangular tubercle, intimately contacts the deep pole of the mentioned neurons. In fishes the *tubercle of connection* is even shorter and simpler, adopting the form of a triangular mass without projections as is shown in figure 27.

The recent investigations of R. SEGARRA (1), demonstrate that in the reptile, where the triangular tubercle sometimes has huge proportions, this simple connection is very striking.

Furthermore, VON LENHOSSÉK has pointed out a similar type of connection in the *ciliary ganglion* of birds and reptiles (2). It is superflous to say that such relationships are strictly by contacts without the least indication of intrasomatic penetration by neurofibrils. The latter, incidentally, are excellently stained in birds and fishes.

C. **Axo-somatic connections by means of large, loose nests from which arise bulbs and terminal feet. ("Endfüssen" of Held).**— The collaterals of the columns of the spinal cord were briefly mentioned by GOLGI (3), and minutely studied by me with the GOLGI (4) as well as the EHRLICH methods (1889-1903). As is well known, they ramify complexly in the gray matter and terminate by forming nests, more or less thick and tight, about the spinal cord cells, especially around the motor neurons. The terminal fibrils end in contact with the cell membrane by means of a varicosity. My description was confirmed by Kölliker (1), and afterwards by van Gehuchten (2), Edinger, Lenhossék (3), Retzius (4), Sala (5), and others. Later the collaterals became a definite acquisition of the science, accepted by nearly all authors.

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(1) SEGARRA: Le ganglion tangentiel ou intercalaire du vestibulaire de certains reptiles. *Trav. du Lab. de Rech. bio.* Tom. XXIV, 1926.

(2) VON LENHOSSÉK: Das ciliare Ganglion der Reptilien. *Arch. f. mikroskop. Anat.* Bd. 80, 1912.

See also: Das ganglion ciliare der Vögel. The same Review. Tom. 76, 1911. (These papers are illustrated with extremely clear and convincing drawings.)

(3) GOLGI: Studi istologici sui midollo spinale. Communication faite au III^{e} Congrès de Reggio-Emilia, 1880. This paper, published in a local medical periodical, was unknown for eleven years by the scholars.

(4) CAJAL: Sur l'origine et les ramifications des fibres nerveuses de la moelle épinière &. *Anat. Anz.* 1890.

But my studies, as well as those of Kölliker, Lenhossék, Retzius, etc., suffered one essential defect: the collaterals and their terminal nests were stained in avian and mammalian embryos a few

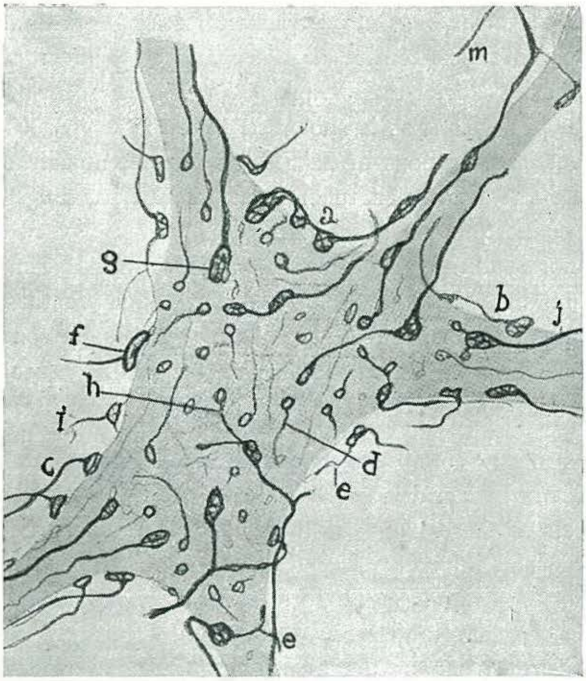

Fig. 28.—Details of the pericellular nest of a motor neuron from the spinal cord of the adult dog. (fourth formula): *a*, *b*, large terminal bulbs; *d*, delicate terminal rings; *e*, fine pale fibrils emanating from a terminal bulb; *f*, *g*, *h*, other bulbs emitting fine, secondary branches.

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(1) Kölliker: Ueber den feineren Bau des Rückenmarks, &. März, 1890. *Vorläufige Mitteilung, Sitz. ber. d. Würzb. phys. med.* Gesell, 8 März, 1890.

(2) Van Gehuchten: La structure des centres nerveux. *La Cellule.* Tom. VII, 1897.

(3) V. Lenhossék: Der feinere Bau der Nervensystems, 1895.

(4) Retzius: *Biol. Untersuchungen.* N. F. Bd. V. 1893.

(5) Sala: La médula de los batracios (in collaboration with me), 1892.

days old, that is to say, in animals in the process of development. Only later was I able to partially demonstrate these fibers in young and adult mammals by means of Ehrlich's method, which unfortunately does not stain the deep gray matter very well. Nevertheless, I was able to show the free terminations of the collaterals upon certain large tangential cells in the substance of Rolando (1). Thus, the true organization of the nests in the adult still remained to be demonstrated.

This was the commendable work which Held (2) pursued in his various researches, employing several methods, but above all the neurofibrillar ones which stain adult neurofibrils. The general result of the important investigations by the scholar of Leipzig was the demonstration that the fine terminal varicosities, described by me and other cultivators of the Golgi method, are present in the adult as thick excrescences applied to the neuronal surface. These swellings, which Held called *Endfüssen* were confirmed by Auerbach (3). who used a special method for this study; by me, utilizing the new technique of reduced silver nitrate (1893) (4); and finally by many other workers, among whom Wolff (5), Holmgren, von Economo and Tello should be named, not to mention the more modern authors.

But the method of reduced silver nitrate (formula 1) and that more recently formulated by Bielschowsky are open to error. Their main fault is that they stain a viscous substance surrounding the neurons, as well as many other objects foreign to the neurofibrils, particularly if recourse is taken to gold toning. I was doubtful of the excessively variable and falacious results of the first silver formula (defects also charged against the excellent BIELSCHOWSKY technique), and for this reason I later formulated some methods which stained intensely and exclusively the *Endfüssen* of HELD (1) but which produced little or no effect upon the neurofibrils. With one of the extremely selective formulas no error is possible because it does not stain the pericellular substance (2), the neuroglia or the GOLGI net and it does not require complementary gold toning.

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(1) Cajal: El azul de metileno en los centros nerviosos. *Revista trimestral micrográfica.* T. I.

(2) Held: Beiträge zur Struktur der Nervenzellen und ihrer Fortsätzen. *Arch. f. Anat. Abt.* 1897.

Ibid: Zur weiteren Kenntnis der Nervenfüsse und zur Struktur der Sehzellen, *Abhandl. d. mathemat. phys. Klasse d. königl. Gesell d. Wiss.* Nr. 11. Bd. XXIX, 1904. (This paper is where Held described the neurofibrillar union between the Endfüssen and the neuronal reticulum.) Such a statement was repeated and enlarged upon in later monographs, particularly in: Zur Kenntnis einer neurofibrillaren Kontinuität im Zentralnervensystem der Wirbelthiere. *Arch. f. Anat. u. Physiol. Anat. Abt.* 1905.

(3) Auerbach: Nervenendigung in den Zentralorganen. *Neurologisches Centralbl.* Nr. 10. 1898.

(4) Cajal: Un sencillo método de coloración selectiva del retículo protoplásmico, etc. *Trabajos del Lab. de Invest. biol.* Tomo II, 1903 Here the neurofibrils of the terminal clubs of Held are shown for the first time.

(5) Max Wolff: Zur Kenntnis der Heldschen Füsse. *Journ. f. Psychol. u. Neurol.* Bd. IV, 1905.

Using this modification of the reduced silver nitrate method, it is observed that the vividly impregnated neurofibrillar foot terminates sharply at the periphery of the neuron, from which it is separated by a thin cuticle, at times visible (fig. 29, f). The bulbs of HELD more or less clearly display a reticular structure with vacuoles or spaces of variable appearance, as was indicated by me, the neurologist of Leipzig and HOLMGREN.

When the nerve cell has not undergone appreciable retraction, the bulbs or rings closely follow the cellular contour which they embrace, and one observes many afferent fibers which exhibit knots in their course and which occasionally send forth fine filaments terminating as rings (CAJAL, AUERBACH, HOLMGREN, etc.). The tigroid appearance of the neuronal outline is perceptible only in the best preparations and if the cell surface is examined with the best objectives (3). The general view is that of a series of discoidal platelets applied to the cell membrane.

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(1) This procedure consists in fixing the pieces in formaldehyde for 36 to 48 hours; washing for 12-16 hours; putting them into ammoniated alcohol (50 cc. of 96° alcohol and 5-8 drops of ammonium) for one day; afterwards, treatment with silver nitrate in the usual manner; finally, reduction in pyro-formol. See CAJAL: Quelques formules de fixation destinées à la méthode du nitrate d'argent. *Trav. du Lab.* &. Tom. V, pg. 215, 1917.

(2) I do not judge here the nature of this specific substance designed to attach the bulbs of HELD to the neuronal surface.

(3) See figure 28, taken from an already old controversial paper. CAJAL: L'hyphotèse de Mr. Apathy sur la continuité des cellules nerveuses entre elles. *Anat. Anz.* Bd. 33, 1908.

See also: Die histogenetischen Beweise der Neuronentheorie von His. u. Forel. Ibidem. Bd. 30, 1907.

But if the section is cut in an equatorial direction the terminal bulb is seen with great clearness. It is intensely impregnated and ends without transition, that is to say, sharply upon the neuronal contour. It is clear that it does not send out perforating neurofibrils to the corresponding motor cell.

As I indicate in figure 29 B, a thick nest of unmedullated fibers of various calibers is sometimes present at a short distance from the cell body. The terminal bulbs are emitted from this nest. However, this arrangement is not constant; it is dependent above all on the retraction of the neuron. If it does not shrink, as in figure 28, the nest does not appear clearly.

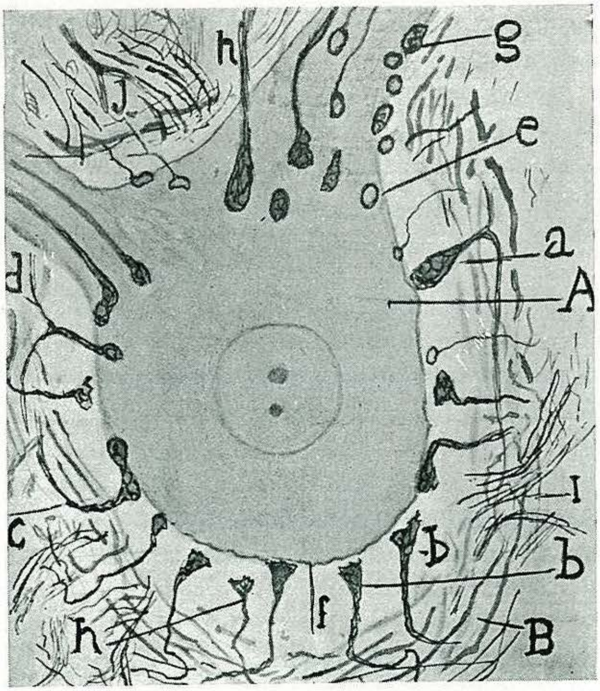

Fig. 29.—Large motor cell from the spinal cord of the adult cat: *A*, neuron stained in an orange tone; *B*, pericellular nests separated by retraction of the neuronal protoplasm; *a*, *h*, giant clubs; *b*, detached clubs; *c*, bilobed club; *e*, *g*, rings in which the pedicles are not stained; *f*, neuronal membrane: *d*. club of passage (figure semischematic). Here are assembled the bulbs existing on two neurons of the same section, very strongly impregnated.

The *Endfüssen* of HELD are late formations. They cannot be seen in the eight day old cat and dog. Instead, one observes loose nests stained by the GOLGI method. It is only in the cat of one month to 45 days that the *Endfüssen* are encountered sporadically and then they are still associated with the typical pericellular plexuses, some of which remain.

The richness of the nests and their terminal feet vary with the size of the neuron. Rare on the small cells, they are more abundant and attain a greater size around the large cells, as for example in *Deiter's nucleus,* in the roof nucleus of the cerebellum and in the motor and interstitial cells of the medulla (1). But this fact is so well known that I shall not dwell on it.

My new observations on the "Endfüssen".—The great importance attributed to the *Endfüssen* of HELD as a decisive argument in favor of the continuity theory has forced me recently to review my old sections and to make new ones using somewhat different methods.

Figure 29 (adult cat) points out some features of these preparations. As I noted some time ago and as is shown here, at first glance one sees various types of bulbs distinguished as much by their size as by their form and structure. Some of these varieties were described by HELD.

In my sections the following species can be distinguished:

1. Extremely fine fibers terminating on the cell body by means of rings. These forms, observed and drawn by me in 1903, have been well presented by HOLMGREN and BIELSCHOWSKY. Some of these rings seem isolated because their afferent fibrils were not stained (fig. 29, g). It is probable that these rings are the visual representations of the fine outline of sectioned bulbs stained with silver.

2. Heavy bulbs in the form of a mallet or cone provided with an interior neurofibrillar net (fig. 29, h).

3. Thick bilobed and trilobed bulbs resting tangentially upon the neuron (fig. 29, C). It is not unusual to observe in the shaft or in the final thickening of these feet the emission of collaterals which terminate as rings, as I indicated some time ago (2) (fig. 28, h).

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(1) They are completely absent in the substantia gelatinosa of ROLANDO and in the nuclei of the columns of CLARKE and BURDACH, all of which suggests that these bulbs represent the termination of a reflex sensory apparatus exclusively for the large motor and funicular neurons.

(2) CAJAL: L'hypothèse de la continuité d'Apathy, &. *Trab.*, &. Tom. VI, 1908.

4. Large bifurcating bulbs, one branch of which is usually not as thick as its fellow (fig. 28, c and 30, c).

5. Extremely heavy bulbs of passage which arise from a fiber coursing at a greater or lesser distance from the cell (fig. 29, a, d).

Some of the *Endfüssen* appear detached, affecting a conic form with one surface parallel to the cell (1). It is not unusual that this surface exhibits some unevenness. The ready separation of these feet from the cell body is significant because it indicates that their adhesion to the membrane is not strong enough to prevent their detachment, as I, TELLO and other authors have already pointed out. It is also striking that this surface, accidentally freed from contact with the neuron, presents no filaments or wavy neurofibrils having the appearance of rootlets (figs. 29, h, b and 30, b).

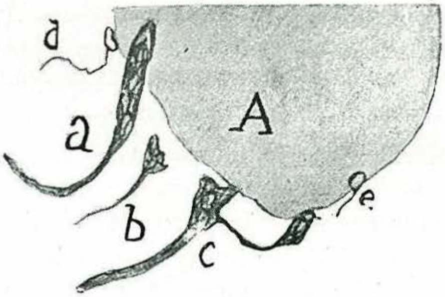

Fig. 30.—Details of clubs of different forms, examined under high magnification: *a*, giant club; *b*, detached conic club: *c*, club which in emitting an accessory one, in terminal rings, provides a peduncle (motor cell of the adult cat).

All these forms, even the finest, are submerged in a mass of neuroplasm unstainable by the neurofibrillar methods but evident with GOLGI and AUERBACH procedures. In my sections the neurofibrils are so intensely stained that gold toning not only would be superfluous but actually detrimental, since it would darken the background and destroy the beautiful contrast existing between the soma (where sometimes one observes many fine, scarcely visible neurofibrils stained rose or orange) and the intense brownish black color of the mallets and rings. On the contrary, in the BIELSCHOWSKY method, toning is necessary. This is a great inconvenience because even if it has the advantage of intensifying the color of the neurofibrils, it also stains the pericellular substance and other matter foreign to the architecture of the neuron. In general, I believe intense simultaneous impregnation of the protoplasmic structure constitutes a disadvantage in the simple interpretation of the organization of the *Endfüssen*. This intense impregnation is almost constant when the ammonium silver oxide method is used.

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(1) ESTABLE, VILLAVERDE and other authors such as TELLO, etc., have seen how the bulbs of HELD are separated very easily from the neurons in various pathological processes. See above all, ESTABLE: Zur Histopathologie der Friedreichen &., *Travaux du Labor.*, &. Tom. XXVII, 1931.

Opposition of Held's school and doubts of Bielschowsky concerning the independence of the "Endfüssen" and rings.—The arguments of the followers of HELD's theory can be reduced to two:

1. The *Endfüssen* do not represent free pericellular terminals, but instead they give off penetrating neurofibrils to the underlying neuronal reticulum with which they are continuous (1).

2. In addition to this type of termination there exists another represented by a superficial pericellular net ("pericelluläres nervöses Terminalnetz" of HELD) which arises from the *Endfüssen*. This nervous net, originally described by AUERBACH (2), extends to the whole or to a part of the cell. According to AUERBACH, the fine reticulum is independent of GOLGI's pericellular net, the non-nervous nature of which has been affirmed by many scholars. In agreement with HELD this scholar believes that the neurofibrillar perisomatic net arises from the base of the *Endfüssen*. But there exists an essential difference between the thinking of these two investigators. According to HELD and his followers, the feet, as we have designated them, send off fine filaments continuous with the somatic framework. AUEBARCH, on the other hand,

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(1) HELD: Zur Kenntnis einer neurofibrillären Continuität in Centralsystem der Wirbelthiere. *Arch. f. Anat. E. Physiol. Anat. Abt.* 1905. Above all consult his book.

See also: H. Held. *Archiv. f. Psychiatrie.* Bd. XLI, 1897. Above all consult his last paper which contains an enlightening resumé of his ideas. Its title is: Die Lehre von den Neuronen und vom Neurencytium und ihr heutiger Stand. *Fortschr. der Naturwiss. Forschung.* N. F. H., 8., 1829.

(2) AUERBACH: *Neurologisches Centralbl.* Nr. 10. 1897. Nervenendigung in den Centralorganen. *Neurol. Centralbl.* 1898. Nachtrag zu dem Aufsatz: Nervenendigung, &., *Neurol. Centralbl.*, 1898.

maintains that such perforating filaments do not exist; rather a continuous transparent cuticle is always interposed between the two nervous reticula.

Although in his last work the histologist of Leipzig did not discuss GOLGI's net, he seems to adhere implicity to his former opinion that the external reticulum is not of a nervous nature but is a dependency of the neuroglia. This theory was also sustained by Donaggio and other authors. The reader notes again that the organization of the terminations of the central nervous system becomes complicated under the presumptuous pen of the reticularists. Instead of accepting the terminal feet as the only mode of interneuronal connections, we have to deal with four types of arrangements: the GOLGI net, independent of the terminal reticulum; the *Endfüssen*, a real and well established entity; the supposed pericellular nervous net; and finally, the union of fibers with the framework of the soma.

All this complicated system of relationships, difficult to demonstrate (except in the case of the terminal bulbs) leads to endless confusion, so much the more so, since each reticularist upholds a different interpretation, in part at least (1). A skeptic might ask whether specific pericellular nerve endings exist, or whether the true idea of HELD would not be to regress to APATHY's and BETHE's doctrine of continuity. According to this theory the ganglionic cell represents a nodal point, endowed with trophic function, interlaced in a diffuse continuous network of conducting filaments. The title of HELD's work, mentioned above, where he alludes to a *Neuronencytium* is suspicious enough. But later I shall discuss the most recent conception of the neurologist of Leipzig and the dangerous reefs upon which his ideas threaten to founder.

For the present I shall limit myself to pointing out the misgivings suggested by the two mentioned interpretations of Held and his disciples concerning the pericellular endings in the spinal cord and medulla. To this end we must submit the descriptions and figures of Holmgren, Held and Bielschowsky to a critical analysis. For the sake of brevity I shall omit here the opinions maintained by other reticularists because they are in general analoguous to those of Held and Auerbach.

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(1) For example, WOLFF, in his special paper (Zur Kenntnis der Heldschen Nervenfüsse. *Journ. f. Psychol. u. Neurol.* Bd. IV, 1904-1905), described and drew a type of pale, spongy network between the foot and the neuron, which is especially visible in the separated Endfüssen. This spongy network, almost imperceptible, would also surround the contour of the foot; it seems to me that it is an artifact produced by an excessively strong silver oxide staining of the coagulated exudates in the perineuronal, space. In our heavily stained preparations such spongy networks have never been shown, either by reduced silver nitrate or by the BIELSCHOWSKY technique which, by the way, is very uncertain when used for the impregnation of the terminal bulbs.

Let me say a few words about Holmgren's old ideas. As can be seen in figure 31 (1), this author not only shows the characteristic foot of Held but also a kind of cone or purveyor of thick, penetrating neurofibrils bound together with the protoplasmic reticulum which is represented coarsely and schematically (2). What necessity is there to comment on such a fantastic arrangement? I shall restrict myself to the statement that in the hundreds of sections intensely stained by diverse formulas, never have I observed such an organization. I suspect that these fibrillar cones have been produced by the retraction of neurons, which draws after it a group of filaments from the *Endfüssen,* stretching them out and dislocating them.

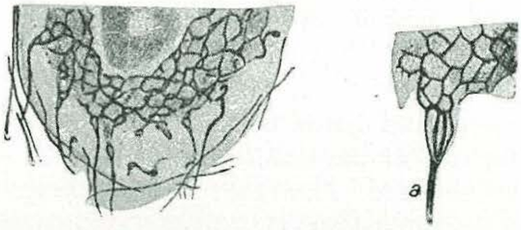

Fig. 31.—Supposed communication between the bulbs of Held and the intracellular reticulum, according to Holmgren (motor cells of the spinal cord) (figures enormously subjective).

The attitude of Held on the problem of his *Endfüssen* terminations is very well known. I have discussed it repeatedly, particularly in my arguments with Apathy.

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(1) Holmgren: Ueber die sogenannten Nervenendfüssen (Held), *Jahrbücher für Psychiatrie,* &., *Neurologie.* XXVI, Bd. 1905.

(2) To tell the truth, in the original drawings of Holmgren the fibrils of these terminal cones are very subtle; obviously, in the picture of Held's monograph which represents them, the great diameter of the mentioned fibrils has been exaggerated, as have those shown in my figure 31, which is a copy of the drawing published by the scholar of Leipzig.

We should remember that according to the Leipzig scholar and his disciples, the feet flow into a double system of neurofibrils: the *pericellular system,* already mentioned, and the system of filaments communicating with the intrasomatic reticulum. As Auerbach indicated, the *net* or *pericellular veil* flows from the base of the *Endfüssen,* which would, therefore, represent the nodal points of this net. In this regard, figure 32, taken from Held's last paper, is most expressive. It shows the pericellular reticulum of a spinal motorneuron to which two terminal feet (*a* and *b*) connect. (The section was prepared with the Golgi method.)

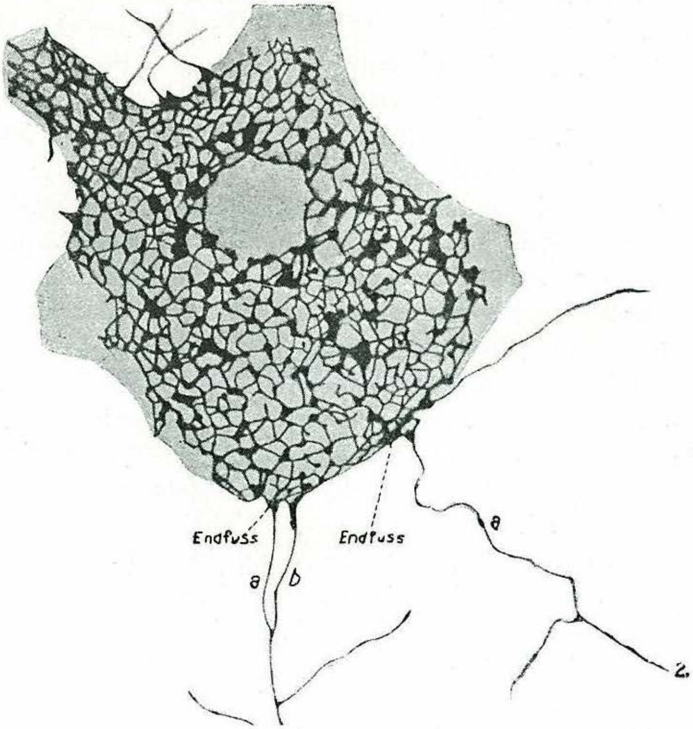

Fig. 32.—Superficial nervous network continous with the *Endfüssen,* impregnated in a motor cell: *a, b,* afferent fibers to the reticulum (from Held).

On various occasions I have discussed the probable causes for the illusion of penetrating neurofibrils (1). We are very likely dealing with accidental arrangements caused by protoplasmic wrinkling. The proximity of the two reticula *(Endfüssen* and intrasomatic, the two being separated by a tenuous membrane invisible with even the highest objectives) makes such confusion possible in these exceptional cases. Personally, I have never observed this continuity between the two neurofibrillar apparatuses in even the best BIELSCHOWSKY preparations. At any rate the best means of avoiding similar mistakes is to have recourse to methods which stain the *Endfüssen* exclusively or almost exclusively. In such sections it is always observed that the reticulum of the terminal bulbs ends sharply at the surface of the neuron. Undoubtedly, one could object that while the intraprotoplasmic framework is not stained and the *Endfüssen* are, it follows that it would be impossible to observe this continuity between the two neurofibrillar systems. Actually this sharp interruption of the staining reaction is a fact favorable to the neuron theory because it indicates that at the level of the membrane there exists an obstacle which is almost always resistant to the reducing agents. On the other hand this fact does not explain well why, at least in some cases, filaments do not emerge from the *Endfüssen* to partially invade the colorless cell body.

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(1) *Anatomischer Anzeiger.* Bd. XXXIII, 1908.

Regarding the marginal veil we should certainly note that such an arrangement, observed by AUERBACH and eventually by HELD (gelegentlich), presents important difficulties. Here are some of them:

a) If the net is as thick as HELD's drawings indicate and if it emanates from the *Endfüssen,* it should be impregnated by the neurofibrillar methods at least in the case of the spinal cord, acoustic ganglia and motor centers of the medulla. Now, all my attempts to stain this net with various formulas of reduced silver nitrate and the BIELSCHOWSKY method have failed. Certainly HELD described and drew (HELD's fig. 10) (1), a reticulated neurofibrillar veil upon a thick dendrite of the spinal cord (anterior horn); but this figure, which presents a Pleiades of terminal rings united by extremely delicate bridges, is not convincing (2).

Many times have I seen groups of closely placed rings, but never anastomotic trabeculae. Empty spaces always appear between the mosaic of rings and plates. The impression one receives when looking at HELD's picture is that he has been led into error by the coloring of some colloidal precipitate deposited among the rings and accidentally united with them.

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(1) See above, the last and repeatedly quoted work by HELD.

(2) Remember our caution about the true form which has a ring-like appearance. It is probably a case, I think, of a cortical layer with a strong affinity for silver.

b) The superficial reticulation described by HELD (fig. 32, silver chromate impregnation) is so similar to the so-called pericellular reticulum of GOLGI, DONAGGIO and other scholars that it is almost impossible to avoid the impression that GOLGI's net and the one reproduced by HELD are one and the same. The pericellular reticulum of GOLGI is considered to be of a non-nervous nature.

c) We should not be swayed by the fact that the neurologist of Leipzig has seen the feet insert into the nodal points of the net. It is well known, as I have stated so many times, that the silver chromate sometimes produces misleading precipitates which adhere and coalesce between neighboring fibers. To accept such rare accidents as the normal preëxisting structure would be very naive. Thirty five years of working almost exclusively with the GOLGI method have convinced me of the importance of so great a cause of error and have made me cautious and suspicious when viewing unusual and peculiar details.

Due to the existence of these and other artifacts provoked by the fixatives —no one of them is perfect— I believe that great prudence is required when interpreting extraordinary forms and rare connections between neighboring structures. One should note in this respect that the reticularists, in their published figures dealing with the *Endfüssen,* chalices, etc., have usually omitted the most common relationships whereas the most peculiar and spurious ones are shown with morbid delight.

Moreover, it would be childish to conceal the fact that in some synapses (those of the acoustic nerve, motor cells, etc.) the intimacy between the component parts of the connection is very great. It is possible to see fields, especially in oblique sections, where the illusion of continuity is excusable. Nevertheless, one shoud depend upon the most common images which are produced when the impregnations are strong and sharp. If not, the subjective factor which is eliminated with such difficulty when using high power will lead to unfortunate obscurities and contradictions. For this reason it has been said that the histological controversies are due more to diametrically opposed psychologies than to unjustified preferences in technique.

Regarding BIELSCHOWSKY's attitude toward the neuron theory, it is noticeable that in his recent papers, where he discusses this problem, he is more cautious than HELD, BETHE, WOLFF, HOLMGREN, etc. Nevertheless, it seems to me that despite his great caution and knowledge of the errors which the neurofibrillar methods can produce, he inclines a little toward the hypothesis of continuity at least in the case of certain special synapses. If one judges by the data presented in his last paper (1) and above all by the appearance of figures taken from his sections, I would say that they have undergone shrinkage and coalescence probably due to fixation in formalin. In one of the figures (57, *a*, *b*. *c* of BIELSCHOWSKY) the bulbs and rings appear free and without continuity with the reticulum of the surrounded cell. In others one observes some confusion and intermingling between the external rings and the intrasomatic reticulum which could give the impression of neurofibrillar penetration. Sometimes (fig. 58) in figures representing the neurons of the *ventral acoustic nucleus* and DEITER's *nucleus*, it seems as though the bulbs of HELD generate a superficial net. What arouses doubt in the mind of the reader is the use of extremely low power or else the drawings are much reduced in the photograph (2).

In any event, none of BIELSCHOWSKY's figures clearly reveal a continuity between the reticulum of the *Endfüssen* and the intrasomatic framework. If I interpret correctly BIELSCHOWSKY's thinking in his last paper, this author leans toward the continuity theory, but only for certain types of synapses. He accepts the neuron theory in principle but he does not raise it to the level of absolute dogma. Although this might not be believed, I defended many years ago a similar position, above all in the case of some interneuronal anastomoses in the sympathetic ganglia.

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(1) BIELSCHOWSKY: Article of the Möllendorff's book. Tom. IV, pgs. 108 and 109. J. Springer, Berlin, 1928.

(2) There are many modern scholars, particularly in the Anglo-Saxon countries and in Italy and France, who cannot be convinced of the existence of such communications. Let us quote, as one of many examples, that of WILLIAM F. WINDLE and S. L. CLARK, who drew the *Endfüssen* under high magnification as being absolutely independent of the neurons. See: Observations on the Histology of the Synapse. *J. Comp. Neur.* Vol. 26, 1928.

The physiologists, among them the illustrious SHERRINGTON, LANGLEY and many others, sometime ago adopted the same position. LEVI and TANZI, and LUGARO, great authorities of Italian neurology, have declared the'r neuronist creed. The skepticism of the scholars toward the reticular theory is absolutely justified.

CHAPTER VII

7.—CLIMBING FIBERS OR LONGITUDINAL AXO-DENDRITIC CONNECTIONS. AXO-DENDRITIC CONNECTIONS BY MEANS OF GEARS.

The most typical example of this first connection is that of the *climbing fibers* which wind along the dendritic arborization of the PURKINJE cells like a vine.

Discovered by me in the avian cerebellum in 1888 (1), and later described in mammals and man in succeeding papers and books (2), they were afterwards noted by nearly all the workers who used the GOLGI methods, as GOLGI, KÖLLIKER (1893), RETZIUS (1892), LUGARO (1895), HELD, ATHIAS (1897), P. RAMÓN and ILLERA. Finally, they were confirmed by those who, from 1904 on, studied the cerebellum with the neurofibrillar procedures and were a powerful contribution in establishing the hypothesis of transmission of the nerve impulse by contiguity.

Here I shall not enter upon a detailed description of the climbing fibers because they are very well known to the neurohistologists. Suffice to say that some relatively thick axons arrive from the white matter, almost always cross through the granular layer without bifurcating and reach the neighborhood of the periphery of the PURKINJE cell bodies where they sometimes intermingle with the baskets. After passing by these, they are incorporated on the heavy ascending shaft of the PURKINJE cells, or, after bifurcating, form more or less complicated plexuses in contact with the various protoplasmic branches (dog and man). After dividing profusely, they contact intimately the heavy dendrites and their secondary branches, avoiding only the finest or terminal ramifications (fig. 33) (1).

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(1) CAJAL: Sobre las fibras nerviosas de la capa molecular del cerebelo. *Revista trimestral de Histología normal y patológica.* Agosto de 1888. (With a lithographic plate.)

CAJAL: Sur les fibres nerveuses de la couche granuleuse du cervelet, &., *Internat. Monatsschrift, f. Physiol.* Bd. VII, Nr. i. 1890.

(2) KÖLLIKER: Handbuch der Gewebelehre des Menschen. B. 2. Erste Hälfte, 1893 (Sechste Auflage) S. 364 und 365.

The whole of this peculiar arborization copies in a simple manner the ramification of the PURKINJE dendrites. Each dendrite may be

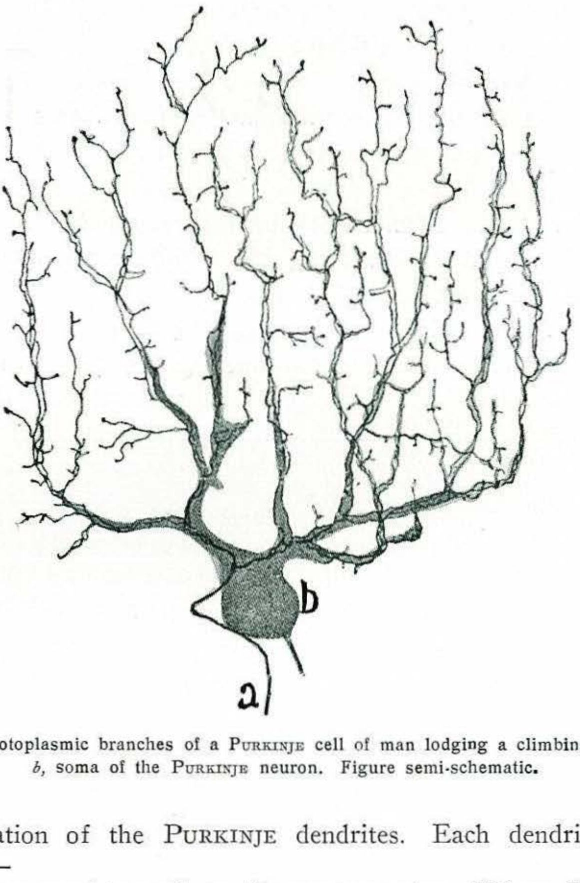

Fig. 33.—The protoplasmic branches of a PURKINJE cell of man lodging a climbing fiber: *a*, fiber; *b*, soma of the PURKINJE neuron. Figure semi-schematic.

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(1) See my complete work on the nervous system. Volume II, p. 65 and following. Paris, 1911 (French translation by Dr. AZOULAY). In this book the reader will find also a resumé on the evolution of these interesting afferent axons, confirmed by RETZIUS and other scholars. See also: CAJAL and ILLERA: *Travaux du Laborat. de Rech.*, &. Tom. V. 1907.

Among the investigators of the early years, consult above all the valuable paper by RETZIUS: *Biol. Untersuchungen.* N. T. Bd. III, 1892. The Swedish scholar not only confirmed my description of the climbing fibers, but also adduced interesting data on their evolution.

bordered by one, two or more nerve branches. The highest and finest end freely with or without a terminal varicosity (1).

It is obvious that the complexity and extension of this nerve arborization is related to the richness of the PURKINJE dendritic system.

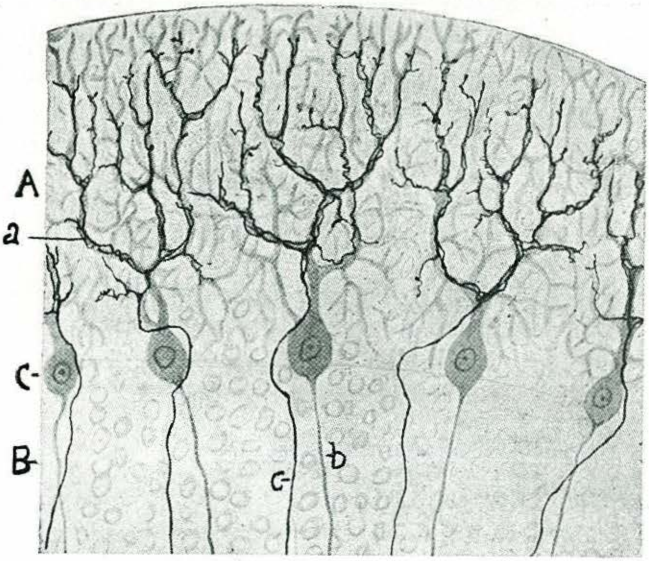

Fig. 34.—A portion of a transverse section of a cerebellar convolution; *A*, molecular layer; *B*, granular layer; *C*, PURKINJE cells; *a*, climbing arborizations; *b*, PURKINJE cell axon; *c*, axis cylinders arriving from the white substance and ramifying on the dendrites of the PURKINJE cells. Kitten. Figure semi-schematic.

It appears extraordinarily complex in man, where it ramifies over a considerable area. Its appearance is simpler in the cat, dog, rabbit and bird and even more so in the cerebellum of the reptile, as my brother has pointed out. In neurofibrillar preparations of cat and dog, the neurofibrils of both elements of the axo-dendritic connection are stained simultaneously. When such sections are carefully studied it is observed, without the least shadow of doubt, that the filaments of the dendritic reticulum and the constituent parts of the nerve arborization are absolutely independent. On some occasions each dendrite is accompanied by two nerve branches and it is not uncommon to see that the climbing projections wind along and even around the dendritic processes, the course of which they intimately follow. This is shown in figure 35, b.

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(1) The fact of this contact is so clear and evident that even BIELSCHOWSKY himself, who is not noted for his credulity, considered the climbing-fibers as a perfect example of mediating connections. In agreement with RETZIUS and myself, he described them not as isolated ascending fibers but as complicated plexuses. He also observed that in the initial part of their course there are some retrograde fibrils descending towards the neural nests. (These fibrils cover the superior portion of the PURKINJE cell bodies.) This peculiarity, noted by myself and well observed by RETZIUS, seems to me to represent the vestige of an embryonic arrangement. At any rate, it is frequently absent. (See BIELSCHOWSKY, in the Handbuch der mikrosk. Anat. de Moellendorf. Bd. IV, 1929). On the other hand, HELD, who is always hunting for syncytia, assumes without proof that there exists a union between the climbing fibers and the PURKINJE cells by means of neurofibrils. Figure 7 shows the eventual concourse that the climbing fibers may take with the baskets around the PURKINJE cell bodies.

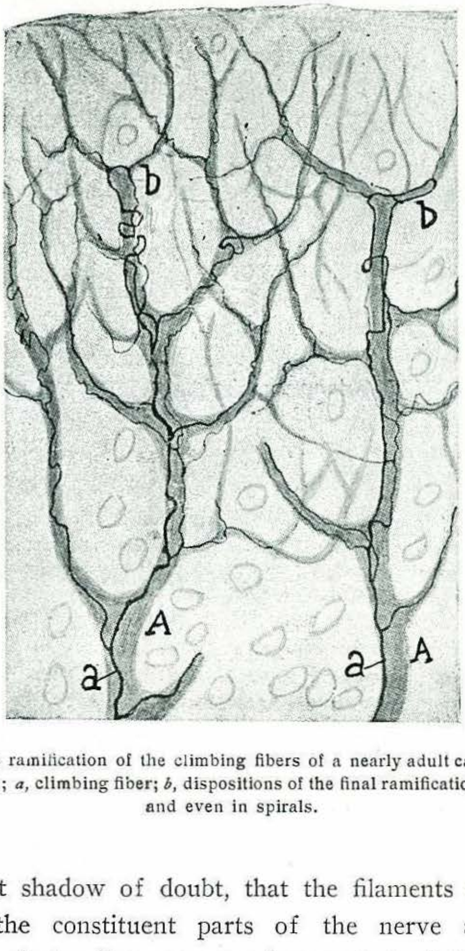

Fig. 35.—Details of the ramification of the climbing fibers of a nearly adult cat: *A*, ascending branch of the Purkinje neuron; *a*, climbing fiber; *b*, dispositions of the final ramifications of this fiber in loops and even in spirals.

where a portion of the nerve arborization has been drawn under high power.

This is a case of connection by contact so evident that the reticularists have generally maintained a prudent silence. Here the contact is seen with absolute certainty. The very few authors who have assumed the existence of connecting bridges between the dendritic framework and the climbing fibers have either suffered a lamentable illusion or they have been victims of distorted subjectivism. I have persistently searched for these supposed anastomoses in the best, BIELSCHOWSKY preparations as well as in those obtained with the strongest and most selective formulas of reduced silver. My failure to find them has been complete, even more, I believe that if such anastomotic bridges did exist, they would escape, because of their thinness, the resolving power of the strongest objective (Zeiss, 1.30-2 mm.).

Furthermore, there is the evidence which pathology reveals. As I have demonstrated in traumatic degeneration of the cerebellum, there is a complete destruction of the PURKINJE cells and a persistence of greater or lesser duration of the climbing fibers. On the contrary, as sometimes happens in chronic pathological processes of the human cerebellum, the climbing fibers are destroyed while the PURKINJE neurons remain. I shall mention some examples of these significant phenomena in the second part of this paper.

Climbing fibers of the sympathetic ganglia; thicket-like terminations.—As a variety of this type of dendro-axonal synapse mention should be made of the one established between the occasionally spiral prolongations of the nerve fibers which come from the spinal cord (preganglionic fibers) and the long dendrites of the sympathetic cells (man, dog, solidipeda). These fibers, described by me some time ago (1), have been well analyzed by CASTRO (2) in is classic work on the structure of the human sympathetic system in which he studied the normal and pathological vertebral sympathetic chain and other visceral centers.

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(1) CAJAL: Las células del simpático del hombre adulto. *Travaux du Laboratoire de Rech. biol.* Tom. IV, 1905-1906.

(2) CASTRO: Evolución de los ganglios simpáticos vertebrales y prevertebrales, etc. *Trabajos,* etc. Tomo XX, 1922.

These works also merit inspection: MARINESCO *(Revue neurologique.* Vol. 8, 1906). SALA, MICHAILOW, MÜLLER. RANSON, ACHÚCARRO, ARCAUTE, BLONDI, BILLINGSTEIN, TERNI, LEVI and many others which are discussed in the extensive, well documented paper of CASTRO.

In addition to the possibly abnormal *spiral fibers* which have their origin in the spinal cord, mention should be made particularly of the great number of free arborizations of the *rami communicantes* which terminate by spreading out into small branches. These branches, after traveling along the bundles or tract of the dendrites, lose themselves in the thickness of these same fascicles, forming very complicated plexuses. Furthermore, I believe that these nerve ramifications upon the dendritic fascicles or the thickets of the dendrites (following the expression of CASTRO) represent the most common and typical manner of preganglionic terminations (1). For this reason I shall not discuss here the *pericellular nests* and neurons with coronal appendages which some authors consider pathological or atypical forms (paraphytes). CASTRO also is inclined to accept this opinion. He affirms that cells with a dendritic crown (my first sympathetic type) are very uncommon in children and young men.

Concerning the perisomatic nests he admits two kinds: the *typical,* in intimate relationship with the cell and formed by the arborization of preganglionic axons; the *atypical* or pathological, formed by skein-like ramifications from other sympathetic neurons. Because of the great difficulty in distinguishing between these two kinds of nests I believe that all or almost all are atypical. In formulating this opinion I take into account their rarity in normal material (as already mentioned by CASTRO and others) and their exceptional presence in the normal adult cat and rabbit (EHRLICH and neurofibrillar preparations).

The perisomatic nests were observed by DOGIEL and me (2), only

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(1) See also my theory of the *normal pathology,* developed in some of my papers, for example, in the treatise on regeneration and degeneration. Tom. II, 1914 pgs. 82 and following.

(2) La Degeneración y Regeneración del sistema nervioso. Tom. II, 1913. This edition appears in an enlarged and improved English edition: Degeneration & Regeneration of the Nervous System. Dr. R. May, Oxford University Press, 1928.

Unfortunately, all the modern authors do not know the Spanish edition of this work, because it has been out of print for some years. (MÜLLER, MICHAILOW, SALA, BIONDI, LEVI STÖRN, etc.). For this reason they have attributed to me some interpretations which were acceptable in 1905 when I described the atypical forms of the sensory ans sympathetic ganglia of man. However, these interpretations became unacceptable after NAGEOTTE, MARINESCO and several authors sporadically and infrequently in completely normal human ganglia from cases of accidental death. On the contrary, their presence is not unusual in the human superior cervical ganglion and in that of the mature and aged higher mammals.

But leaving this incidental question, it is important to emphasize that in my numerous sections of the sympathetic chain I have never observed a typical or an atypical *preganglionic fiber* in continuity with the dendritic reticulum or with that of the cell body. Neither has CASTRO been fortunate enough to find them. But about this matter, as well as the fantastic ideas of STÖHR, I shall speak on another occasion.

8.—AXO-DENDRITIC CONNECTIONS BY GEARING (MOSSY FIBERS, INSECT RETINA, ETC.).

A. **Gearing between granule and mossy fibers.**—The prototype of this connection is found in the relationship established between the rosette formation of the mossy fibers in the cerebellum (cerebellar glomeruli) and the digitiform arborization of the dendrites of the granule cells. As has been known since my investigations of 1888 (1),

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including myself obtained the same abnormal forms in grafted and injured sensory ganglia of laboratory animals.

Moreover, the spirals, nests and atypical forms of the sympathetic cells are very abundant in tabes dorsalis and other pathological states of the nervous system, as CASTRO has observed. My first descriptions of human ganglia were made partially from diseased nervous system.

(1) CAJAL: Estructura de los centros nerviosos de las aves. *Rev. trimestral de Histología normal y patológica.* Tom. I, 1888.

CAJAL: Sur les fibres nerveuses de la couche granuleuse du cervelet, etc. *Intern. Monatschr. f. Anat. u. Physiol.* Bd. VII, 1890.

Many of the authors who used the GOLGI method confirmed them. Let us cite KÖLLIKER, VAN GEHUCHTEN, RETZIUS, LUGARO, FALCONE, ATHIAS, etc. Among those who used the EHRLICH method mention should be made of: DOGIEL, MEYER, and myself. The neurofibrillar technique was used for the first time by me, BERLINER, BIELSCHOWSKY and WOLFF, etc. Recently, HORNE GRAIGIE made a comparative study of the mossy fibers by means of a special silver nitrate formula. (Note on the morphology of the mossy fibers in some birds and mammals. *Travaux du Laborat.*, etc., tom. XXIV, 1926.) Finally, in recent years I myself have made a minute investigation of the cerebellar glomeruli and their components. (Sur in the gray matter of the cerebellar cortex one constantly observes the arrival of certain thick ramifying fibers characterized at various intervals by tuberous excrescences which stain by the GOLGI, EHRLICH and neurofibrillar methods. These excrescences, or rosettes send off short unmyelinated branches which either terminate or give off collaterals.

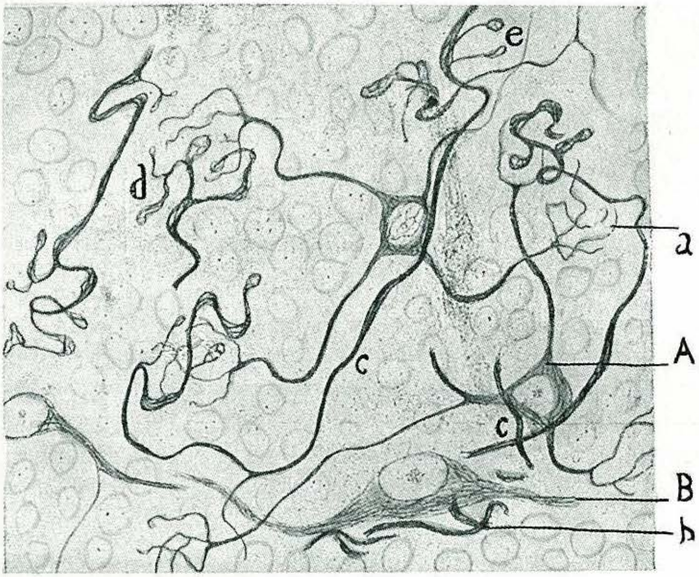

Fig. 36.—Granule cells and mossy fibers of the cerebellum of the adult cat: *A*, granule cell; *B*, star cell or GOLGI cell; *a*, terminal dendritic ramification of a granule cell; *d*, terminal arborizations of the mossy fiber.

The terminal fibers form heavy arborizations even in the preparations stained by some formulas of the neurofibrillar method fig. 5, A).

When the rosette formation is compared with the digitiform dendritic configuration of the granule cells, it is impossible to reject the idea that the two types of ramifications constitute a synapse by gearing. The two elements of the articulation reside in the granular layer in certain special islets characterized by their pale and very granular appearance and by their lack of nuclei (NISSL method). Figures 36 and 37 demonstrate

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les fibres mousseuses et quelques points douteux de l'écorce cérébélleuse. *A book in honour of Prof. Tanzi*, 1926, and *Travaux du Laborat.*, etc. Tom. 24, November, 1926.

well the most salient features of this axo-dendritic relationship which was first brought to attention by me (1), and confirmed by Held (2), and other neurologists such as Hill, Berliner, Bielschowsky and Wolff (3), and Meyer.

If one would suppose, on the basis of the neurofibrillar preparation, that the cerebellar glomeruli are simply territories of convergence and contact between the neurofibrils of both factors of this axo-dendritic gearing, he would be mistaken. Actually, the arrangement is much more complex. Each granular cell ramification —and there are many converging upon the same glomerulus, just as there are at times several afferent rosette formations— possesses a relatively thick plasmatic sheath. For their part, the rosette formations of the mossy fibers are also enveloped by a heavy neuroplasmatic coat. Furthermore, we should add a third element which in all probability participates, namely, the terminal

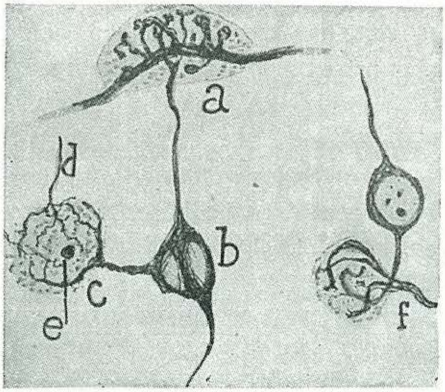

Fig. 37.—Some examples *(a)* of the association of dendrites of the granule cells with lateral projection of the mossy fibers: *b*, body of granule cell with its neurofibrils; *d*, fine fiber probably coming from the axon of Golgi cells. Figure semi-schematic.

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(1) Cajal: Croonian Lecture. London, 1894.

Cajal: El azul de metileno en los centros nerviosos. *Rev. trim. microgr.* Vol. I, 1896.

(2) Held: Beiträge zur Struktur der Nervenzellen. etc. Dritte Abhandlung. *Arch. f. Anat. und Physiol. Anat Abteilung,* 1897.

(3) The figures of mossy fibers, published by Bielschowsky and Wolff seem to me too confusing. (Bielschowsky u. Wolff: Zur Histologie der Kleinhirnrinde. *Journ. des Psychol. u. Neurologie* (1904-1905). Tafell II, fig. 16.

axonal fibrils of the *large stellate cells* of GOLGI (stellate cells of the granular layer). That the glomeruli have an architecture of great complexity is indicated in figure 38, obtained with a most effective neurofibrillar formula. Let us note that the glomeruli exhibit an intricate plexus, the fibers of which do not proceed exclusively from the mossy fibers and the granule cells. In figure 37 I have drawn two granule cells so that the intricate plexuses of figure 38, B may be understood (1).

To reach this interpretation it is necessary to compare the results of the above mentioned neurofibrillar method with the much clearer and simpler ones obtained with EHRLICH and silver chromate preparations. Thus I shall, in a certain sense, elaborate a combined and syncretic picture capable of representing objective reality in the best possible manner. And even then I should have to add some *pale foundation substance* which seems to serve as a unifying cement for all the factors of this labyrinthic gearing (2).

Needless to say the sections stained by the plasmatic methods do not reveal clear anastomoses, as is also true of the neurofibrillar techniques. Moreover, we should remember that the neurofibrils serve as the skeleton of these expansions and that the three mentioned factors of these synapses possess plasmatic sheaths of varying thickness (3). On the contrary, it is not unusual to observe windows or button-holes in the mossy formations stained with the neurofibrillar procedures; these are never seen when the Golgi method is used. From this fact one would conclude that these windows are only present in the fibrillar skeleton and not in the neuroplasm of the arborization. Button-holes are frequent in pathological mossy formations.

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(1) Let us note that the appearance of the glomeruli varies very much with the formula used. Compare figure 36, obtained with the first formula with that in which the procedure of hydroquinone was used (figure 38).

(2) The participation of the nervous ramification og the GOLGI cells in the glomeruli had already been observed by me some time ago in silver chromate preparations. Its presence is one of the strongest obstacles which the observer will encounter in forming a correct interpretation of the pictures obtained with the neurofibrillar methods which at time stain only the terminal nerve fibrils. See CAJAL: Histologie du système nerveux de l'homme et des vertébrés. Tom. II, 1911, pág. 45, figures 30 and 31.

(3) BIELSCHOWSKY, who encountered the same difficulties as myself in investigating these gears, with good reason states that he does not know whether the components in this articulation really touch each other or whether an intercalated substance, which escapes our analysis, exists between them. In reality, as I have already said, both this substance and the terminal ramifications of the GOLGI cells are present.

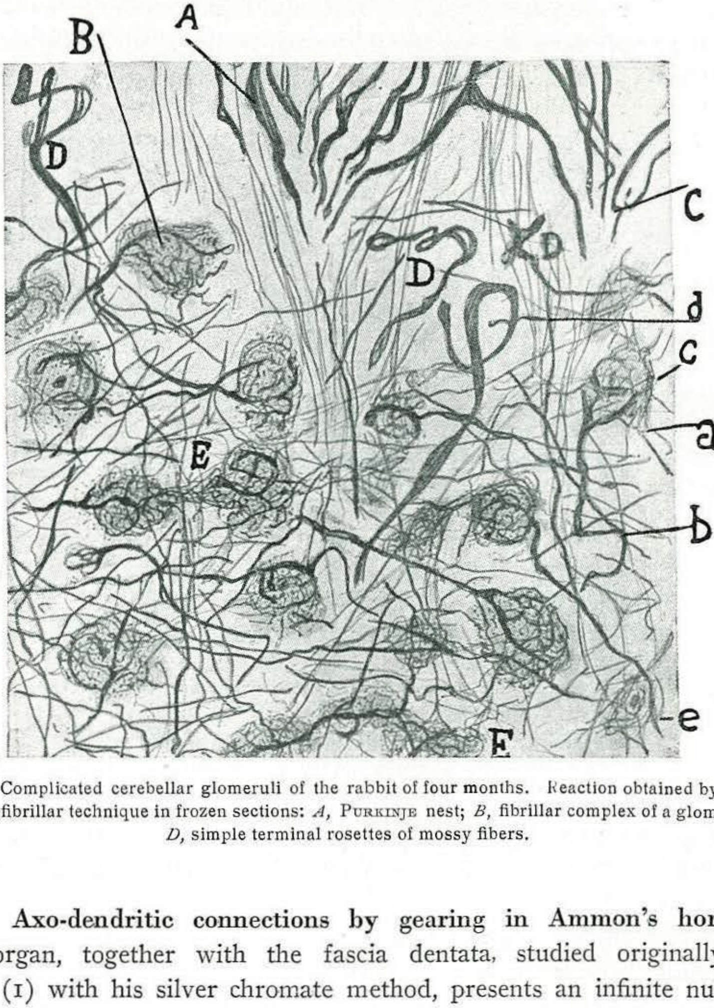

Fig. 38.—Complicated cerebellar glomeruli of the rabbit of four months. Reaction obtained by means of a neurofibrillar technique in frozen sections: *A*, Purkinje nest; *B*, fibrillar complex of a glomerulus; *D*, simple terminal rosettes of mossy fibers.

B. **Axo-dendritic connections by gearing in Ammon's horn.**— This organ, together with the fascia dentata, studied originally by Golgi (1) with his silver chromate method, presents an infinite number of convincing arrangements of connection by contact, which, incidentally, have been very little if at all studied since the appearance of my extensive work on their fine structure (1).

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(1) Golgi: Sulla fina struttura degli organi del sistema nervoso. Milano, 1886.

This is not the place to enumerate nor to summarize these most interesting relationships. My intention is to call to mind once again a highly interesting case of connection by gearing. For greater clarity, a schematic representation of it is shown in figure 39, B, C.

Let us note how the fine axons arising in the small pyramids of the *fascia dentata* thicken progressively when descending to the subjacent field of *Ammon's horn* and how they emit numerous excrescences similar in principle to those of the mossy fibers. When these delicate axons arrive at the region of the *large pyramids* of *Ammon's horn* they enter into intimate contact with certain thick appendages and irregular collaterals arising from the shaft of these neurons, thus forming a

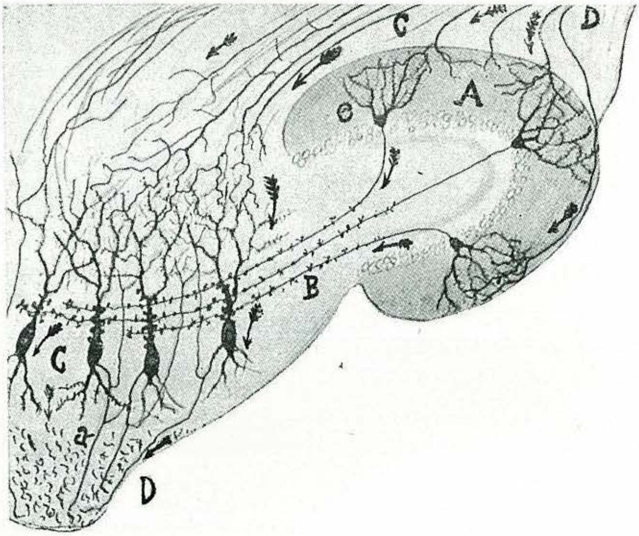

Fig. 39.—Schema to show the connection established between the granule cell axons of the *fascia-dentata* and the large pyramids of AMMON's horn (inferior region of the latter): *A*, molecular layer of the *fascia-dentata; B*, axon of granule cell; *C*, large pyramids; *D*, fimbria; *c, D*, afferent fibers arriving from the secondary olfactory centers; *a*, axon. The arrows indicate the direction of the currents.

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(1) CAJAL: Estructura del asta de Ammon. *An. de la Soc. Española de Hist. natur.* Tom. XXII, 1893. (There is a german translation by Prof. KÖLLIKER in *Zeitschr. f. will. Zoologie.* Bd. LVI, 1893.)

tight geared synapse (C). In this manner the axon of the *granules* of the *fascia dentata* enter into a transversal relationship with a great number of pyramids. The mossy fibers always stop in the region of the radial trunk of these pyramids; never do they reach the *fimbria* or extend to the superior part of *Ammon's horn* (region of the *small pyramids).* I have observed this connection with the GOLGI as well as with the EHRLICH method. The neurofibrillar procedures stain well the pyramids and the axons of the granule cells but not the double apparatus of the geared synapse. Perhaps it is formed exclusively by a neuroplasm in addition to an enveloping membrane. GOLGI had already described, in his classic work, this stream of axons arising in the *fascia dentata* but this author erroneously supposed that it continues through the white substance of the fimbria (fig. 39, D).

C. **Gearing connections in invertebrates.**—The most beautiful and varied arrangements of gearing between dendritic branches and axonal ramifications are found in this group of animals, particularly in insects (CAJAL and SÁNCHEZ) and in crustaceans and insects (HANSTRÖM). One who has not studied the retinal ganglia of *hymenoptera* with the GOLGI method cannot have an idea of the enormous number of neurons which integrate the retina, or of the surprising variety and delicacy of their forms. It seems that nature has attempted to show us in the insect nervous system, particularly in that of the *fly* and *hymenoptera,* how in minimum space it is possible to organize a maximum of fine and subtle structures, perfectly compatible with the highest manifestations of instinct and defensive reflexes. Obviously the great delicacy of the mentioned region is such that it is possible to study it only with the best apochromatic oil objectives (ZEISS 1.30 and 1.40).

Innumerable striking cases of intimate contacts and complicated gearing could be cited (1). Because of the nature of this paper I shall

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(1) I advise those who wish to delve further into this inextricable labyrinth to read the paper I published in collaboration with D. SÁNCHEZ under the title: Contribución al conocimiento de los centros nerviosos de los insectos. *Trab. del Lab. de Invest. biol.* Tom. XIII, 1915, With 85 plates.

It would be equally useful to consult Kenyon's paper, although it is less rich in details and far too brief (The brain of the bee. *Journ. of Comp. Neurol.* Vol. VI, 1896) and to know the more extensive and documented paper of ZAVARZIN on the larva of AESCHNA (Methylene blue method) Histologische Studien limit myself by mentioning only the connection existing in the deep nucleus of retina *(intermediate and internal retina)*. This synapse occurs between the cells, which have been determined to be homologous, respectively, with the *vertebrate rods* and *bipolar cells,* and the cells

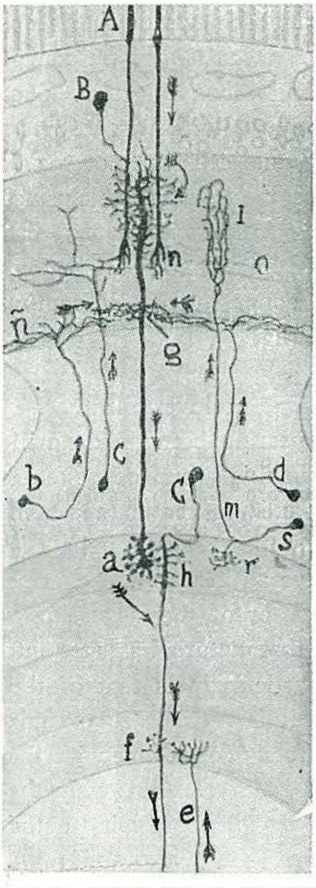

Fig. 40.—Schema of the course of the currents through the retina of an insect. (Bee): *A*, rods; *B*, monopolar cells; *n*, terminal plume of the rods; *C*, ganglion cell; *a*, *h*, articulation between the giant monopolar plume and the collateral branches of a ganglion fiber; *f*, *g* basal dendrites designed to receive centrifugal impulses; *e*, long centrifugal fiber.

über Insekten. *Zeitsch. f. wiss. Zoologie,* Bd. 97, 1811-1814. Finally let me mention the interesting book of HANSTROM on the nervous system of the arthropods, based on the examination of rich and varied materials.

corresponding to the ganglion cells. In figure 40 a small part of the bee's retina, and in figure 41 a typical organization of the retina in the same bee, are shown.

Figure 40 is only a partial reproduction because the presentation of the whole would have required me to make not one but several drawings of huge dimensions (1). Let us note in *B* the geared connection between the inferior pole of the rods and the first *monopolar* neuron (corresponding to the vertebrate *bipolar* cell) and in *a* and *h* the geared synapse between the terminal nerve branches of the latter cell

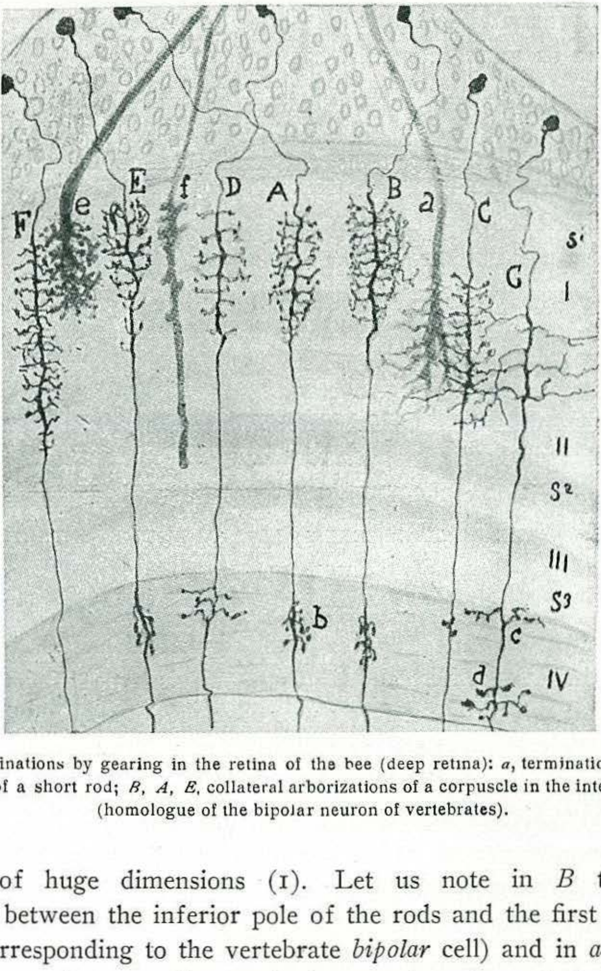

Fig. 41.—Terminations by gearing in the retina of the bee (deep retina): *a*, termination of a long rod; *e*, termination of a short rod; *B*, *A*, *E*, collateral arborizations of a corpuscle in the intermediate retina (homologue of the bipolar neuron of vertebrates).

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(1) A number of such large plates are found in the work (cited above) in which I collaborated with D. SÁNCHEZ.

and the dendritic collaterals of the third monopolar neuron (corresponding to the vertebrate *ganglionic* cell). The organization is somewhat different in the muscidae and other insects. A remarkable detail of the visual neurons of these arthropods is the fact that the soma and the fine initial portion of the neurons do not take part in conduction. Another surprising peculiarity to one who has studied exclusively the neurons of vertebrates is that each single expansions of the nerve cell possesses two or more segments, each of which is destined to contact different axonal systems. Some of these successive segments, bristling with collateral or terminal rami, connect with centrifugal fibers of which the insect retina contains a formidable number. Gearing by small transversal branches is also observed in cephalopods, as VON LENHOSSÉK (2), and I have demonstrated (3), I believe it unwise to detain ourselves here examining the numerous examples of connections by gearing which appear in the retina of cuttlefish, squids, etc.

CHAPTER VIII

9.—CRUCIFORM AXO-DENDRITIC CONNECTIONS OF GREAT LENGTH. MOLECULAR LAYER OF THE CEREBELLUM AND THE INTERPEDUNCULAR NUCLEUS.

A. **Cruciform connections in the cerebellum. Relationship by means of plane, parallel arborizations.** — This type of interneuronal relationship, described by me many years ago (1889, 1890) and confirmed by many workers (KÖLLIKER, VAN GEHUCHTEN, RETZIUS, etc.), is today very well known. For this reason I shall be brief in my description and refer the reader to the accompanying diagram.

The characteristics of such synapses are: with respect to the cells, the more or less perfect flattening of the dendritic branches, and with respect to the terminal filaments, their enormous length, smooth aspect,

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(1) V. LENHOSSÉX: Untersuchungen am Schlappen der Cephalopoden. *Arch. f. mikrosk. Anat.* Bd. XLVII, 1896.

(2) CAJAL: Contribución al conocimiento de la retina y centros ópticos de los cefalópodos. *Trabajos*, etc. Tom. XV, 1917.

free endings at a great distance from their origin and total absence of secondary ramifications. They are unmyelinated, although some workers have supposed the contrary, confusing these fibrils with other types of nerve ramifications.

From the point of view of the synapse in general, it has a disconcerting originality deserving of special mention. Individual reciprocal connection is conspicuously absent here, since the *parallel fiber* touches, as it crosses them at right angles, the spiny portions of thousands of PURKINJE dendrites disposed in series; in turn, each PURKINJE cell establishes relationship with innumerable *parallel fibers* and, consequently, with the granule cells from which they come (see fig. 42, A).

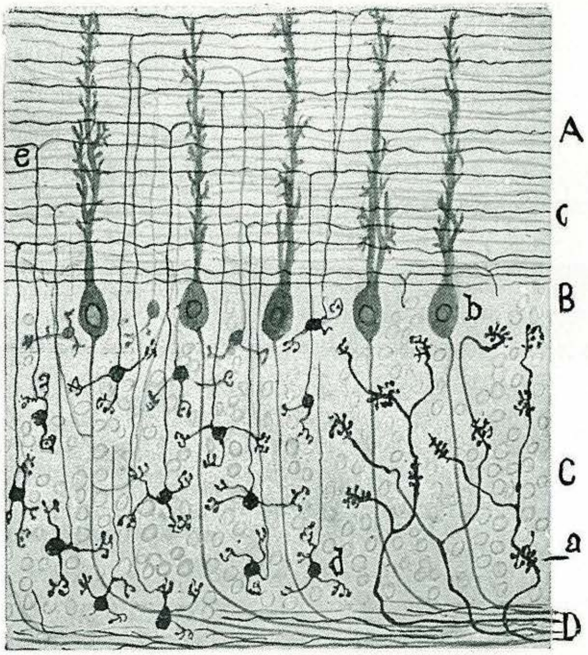

Fig. 42.—Longitudinal section of a cerebellar convolution: *A*, molecular layer; *B*, PURKINJE cell layer; *C*, granule cell layer; *D*, white substance; *a*, rosettes of mossy fibers; *b*, soma of PURKINJE cells; *c*, parallel fibers; *d*, granule cells with their ascending axons; *e*, a division of this axon. Figure semi-schematic.

The parallel fibers of the cerebellum are well stained with the GOLGI method which permits one to determine clearly their origin and relationships. They also have affinity for certain formulas of reduced silver nitrate, this being especially true of those located in the inferior zone of the *molecular layer* (1).

Unfortunately the neurofibrillar reaction, unlike the GOLGI method, does not allow us to determine the type of synapse or even the origin of the parallel fibers, the initial portions of which are not stained by the colloidal silver.

Finally, let us recall in passing another typical case of multiple cruciform connection: that existing in the *interpeduncular ganglion* between the termination of *Meynert's fascicle* and certain cells which are not as flat as the PURKINJE neurons in the cerebellum. This connection, discovered by me in 1894, has been recently confirmed by CALDERÓN (1928) (2) using the Cox method in preparations of the adult mouse.

B. **Axo-dendritic connections by means of plane, parallel plexuses.**—This manner of relationship is found in many nervous organs but is encountered especially in the vertebrate retina.

Let us mention two typical examples which are shown in figure 43.

a) *Contact between the cones and external brushes of the bipolar cells.*—As is shown in figure 43, D, the inferior prolongation of the *cone* ends in the *external plexiform layer* by means of a flat arborization of moderate width. Beneath it another flat ramification, that of the *bipolar cell for the cone* spreads out and becomes interlaced with the first. This disposition is repeated at the inferior pole of the same bipolar cell. Here it spreads out in a flat branch which enters into connection with another laminar ramification of dendrites from the ganglionic cells. In birds and reptiles (fig. 44, A) the horizontal contacts between the mentioned neurons are observed at two or more levels. This is due to the fact that each element of the connection

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(1) All the hydroquinine formulae (fixing the sections in formalin) impregnate in mass the parallel fibers in the inferior half or one-third of the molecular layer. The same occurs, although with less constancy, with the block staining procedure and at times with the BIELSCHOWSKY method.

(2) L. CALDERÓN: Sur la structure du ganglion interpédunculaire. *Travaux du Lab. de Rech. biol.* Tom. XXV. 1927-1928.

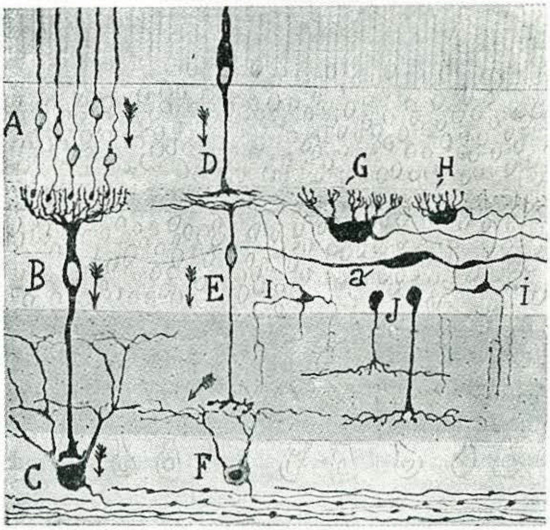
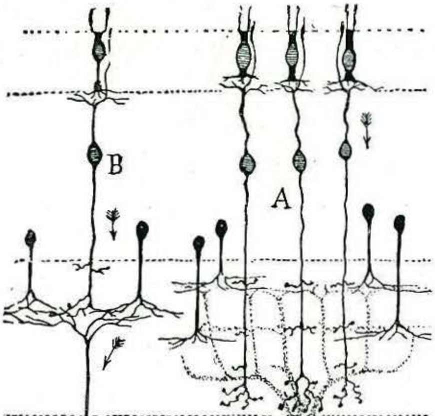


Fig. 43.—Section of the retina of the perch. Semi-schematic figure designed to show the principal results of my researches; *A*, *B*, *C*, specific currents from the impression received by the rods; *D*, *E*, *F*, currents of the excitation received by the cones; *G*, *H*, morphology of the horizontal cells; *a*, *i*, specific elements of the retina of fishes.



Fig, 44.—Schema showing the connections between the neurons of the retina of birds and the course of the nerve impulse: *A*, bipolar cells terminating on the soma of ganglion cells; *B*, short bipolar cells incorporated in the current from the cones.

possesses two or more collateral ramifications which emerge at right angles, or almost so, from the descending prolongations of the bipolar, cells and from the ascending projections of the ganglionic cells. The latter branches are sometimes very numerous.

b) The second example of this type of connection (fig. 44, R, d) is represented by the contact between the *descending terminal brush* of the amacrine cells on the one hand, and the *ascending* branches or certain complex ganglionic cells on the other. This synapse occurs in various planes of the *internal plexiform* layer. It is superfluous to say that definite anastomoses between the two types of contacting branches are not encountered with either GOLGI or EHRLICH methods. The sporadic anastomoses presumed by DOGIEL, RENAUT and others are explained as accidental precipitates of methylene blue deposited between some of the proximate branches of the mentioned elements. A careful and persistent study of these supposed unions by continuity, using silver chromate and EHRLICH methods, has convinced me that such anastomoses are artifacts (1). It is just as impossible to observe organization by continuity in the retina of the chicken embryo.

CHAPTER IX

10.—TERMINATIONS UPON CIRCUMSCRIBED CELLULAR PLEIADES. CONNECTIONS IN THE THALAMUS. COMPLEX CONNECTIONS IN THE CEREBRAL CORTEX.

A. **Terminal arborizations in the thalamus.**—Here I shall limit myself to mentioning only two typical examples pertaining to the thalamus. Similar case are observed in some centers of the medulla oblongata and midbrain. For their study the reader should consult my extensive work on the nervous system.

a) *Sensory terminations.*—These endings are present in the *lateral ganglion* of the thalamus where they occupy an almost spherical area. Here the axons constituting the *sensory pathway* or *internal lemniscus* spread out in thick, independent branches among which lie the bodies and dendritic processes of the cells generating the superior sensory pathway or *thalamo-cortical* projection. As is shown in figure 45 (24 day old mouse) the final nervous arborization is well limited and varicose in appearance. It represents the ramification of a single axon of the internal lemniscus. If space would permit I would also describe the arrival of certain centrifugal axons from the cerebrum, apparently ending by means of free arborizations in each cellular *Pleiades*.

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(1) See CAJAL: Nouvelles constributions à l'étude histologique de la rétine et à la question des anastomoses etc. *Journal de l'Anatomie et de la Physiologie* 1896. (Avec 4 planches.)

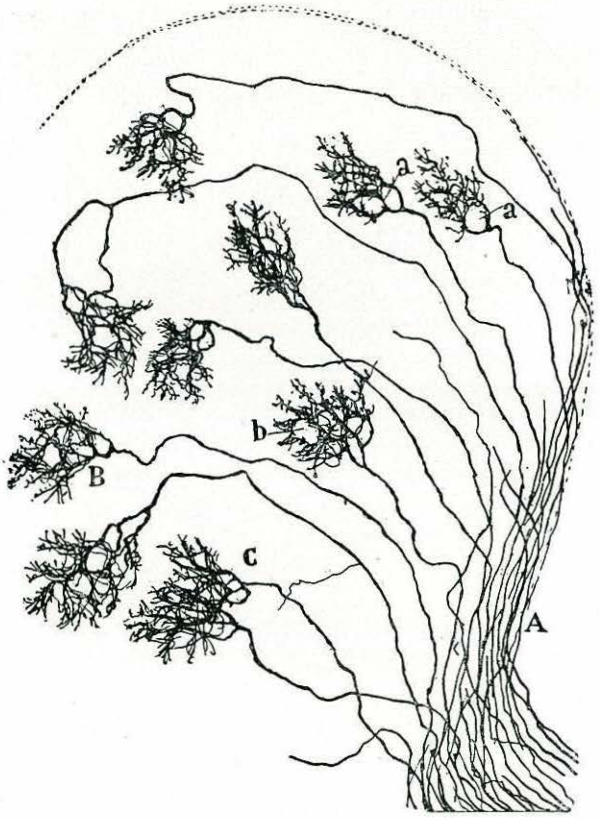

Fig. 45.—Portion of the lateral nucleus of the thalamus where the sensory fibers terminate. (ZEISS Obj. C. 25 day old mouse.) GOLGI method, double impregnation: *A*, sensory path; *B*, terminal arborization of a fiber; *C*, two fibers intermingling their ramifications; *a*, *b*, cavities in the arborization reserved for cells (saggital section).

These nests do not have *Endfüssen* of HELD. I have succeeded in impregnating this arborization, although only imperfectly, since the last terminal fibrils always escape the neurofibrillar reaction.

b) *Terminations in the habenular ganglion.*—The second example also pertains to the thalamus. It is represented by the *habenular ganglion* which is supposed to be dependent upon the olfactory system. The thick afferent fibers belong to the pathway of the *stria medullaris.* As is shown in figure 46 (20 day old rabbit) each ramification is extremely dense and has free spaces for three or more cells of the *internal portion* of the *habenular ganglion.* The neurons which receive the olfactory impulse possess peculiar morphological characteristics. They are nearly all provided with a thick tuft, resembling a bush, and from their small bodies emanates a fine descending axon incorporated into Meynert's *fasciculus.*

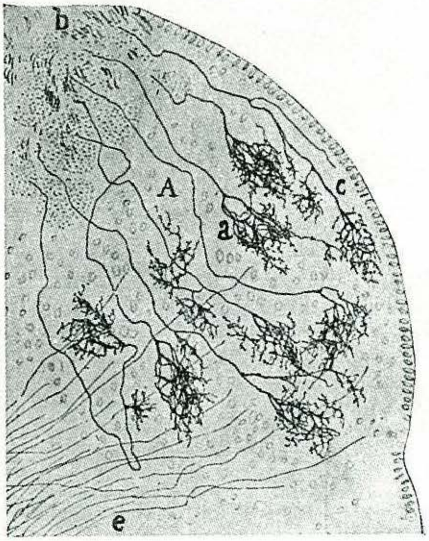

Fig. 46.—Free arborizations disseminating across the internal habenular nucleus *(A)* and coming from the olfactory pathway designated *stria medullaris (b).*

c) *External geniculate body.*—The *visual arborizations* in the *external geniculate body* are equally interesting. They were originally described by P. RAMÓN (1890, '91), me (1894) and TELLO (1904). Here it is observed that each fiber coming from the retina contacts linear *Pleiades* of neurons by means of multiple nests. If space would allow I would also discuss here the terminal ramifications of the visual fibers in the *optic lobe* of fishes and reptiles, masterfully described by P. Ramón (1890), as well as the homologous structure in birds, studied first by me (1889) (1), and then by van Gehuchten and other workers.

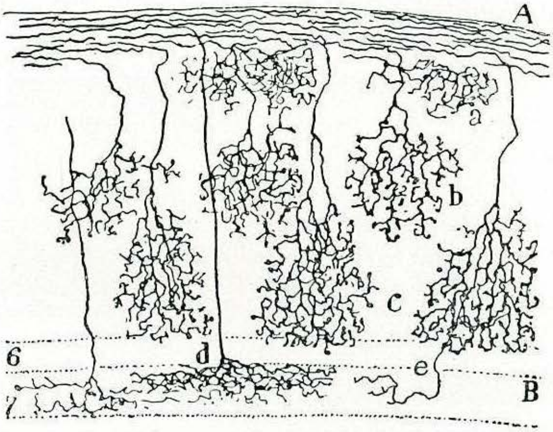

Fig. 46 bis.—Different tiers of optic arborizations in the cortical gray of a bird optic lobe: *A*, fibers from the retina; *a*, *b*, *c*, free arborizations.

Here, as in mammals, the arborizations are diffuse, each one embracing a special territory in which lie the dendrites of numerous fusiform neurons (fig. 46 bis).

B. **Complex terminations in the mammalian cerebral cortex.**— Numerous scholars have labored with this problem which is the most arduous and difficult presented to us in the study of the fine structure of the nervous system. Primacy in this endeavor is deserved by Golgi (1885) who employed his famous silver chromate method (2).

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(1) Cajal: Sur la fine structure du lobe optique des oiseaux, etc. *Journ. inter. d'Anat. et de Physiol.* Tom. VIII, 1891.

(2) Golgi: Sulla fina anatomia degli organi centrali del sistema nervoso. Millan, 1885. In Spain the black reaction has been assidously applied by my brother, P. Ramón, on lower vertebrates (1890-96); by Tello (1904) on the terminations of the external geniculate body of the cat; by Calleja on the frontal olfactory region of the human cerebrum (1893); by Lorente de Nó on the posterior portion of the mouse cerebrum (1923-24). Villaverde (1924) and

Several of is disciples continued the work, among them MONDINO (1886), MARTINOTTI (1890), VERATTI (1897), LUGARO (1893) and various others. Outside of Italy, KÖLLIKER (1890), CAJAL (1890, '91, '96, etc.), RETZIUS (1891, '94), VAN GEHUCHTEN, P. RAMÓN, K. SCHAFFER (1897), etc., used the same method and confirmed many of the findings of the school of Pavia. I omit here a description of the fine structure of the neurons (NISSL bodies, neurofibrils, myelin, etc.). These details are studied with methods which do not stain the central terminations and consequently cannot throw any light upon the problem of intercellular connections.

I have already mentioned the enormous difficulty in the clarification of interneuronal synapses presented to us by the network of the gray substance. This problem has not escaped the attention of any neurologist. It resides in the fact that in the thalamus, cerebellum, olfactory bulb, retina and other structures, the nerve arborizations are located in special territories or layers where they connect with circumscribed *Pleiades* of neurons. On the other hand, in the cerebrum (also in the *external geniculate body* but not in *Ammon's horn)* the collateral and terminal nerve ramifications attain an extraordinary length and diffusion as well as a great variety of directions. The entirety of a field completely impregnated by the GOLGI method is comparable to a black felt with free spaces corresponding to unstained glial or nervous elements.

Concerning the neurofibrillar methods, so effective in the fetus and very young animals, they are disappointing in adult or nearly adult mammals because they frequently stain simultaneously all the medullated and unmedullated fibers as well as the dendrites. To this difficulty is added, due to the thickness of the interstitial plexus, the absolute necessity of cutting thin sections in which the nerve fibers appear mutilated at greater or lesser distances. Figure 47 offers an idea of the exceptional density of such a network.

ESTABLE (1924), respect'vely, have examined the superior cortical regions of the rabbit and of the amphibians.

A detailed bibliography would have required too many pages. To document himself, the interested reader ought to go back, for the older works, to GOLGI, MODINO, VERRATI, CAJAL and KÖLLIKER, VAN GEHUCHTEN, etc. (extensive works and papers) and for the modern works he should take recourse to the monographs of NISSL, BETHE, HELD, CAJAL (1921). LORENTE DE NÓ, etc.

The method of EHRLICH can be of no more use to us since methylene blue ordinarily stains only the dendrites and soma of the large neurons of short axon, sparing the axon itself. I have attained results with the latter only in exceptional cases, for example, in the great fusiform cells of the first layer of the cerebral cortex (1).

One is therefore limited to the GOLGI method and those similar, such as that of Cox. Only the black coloration of the silver chromate furnishes us with correct and expressive, though often incomplete (2),

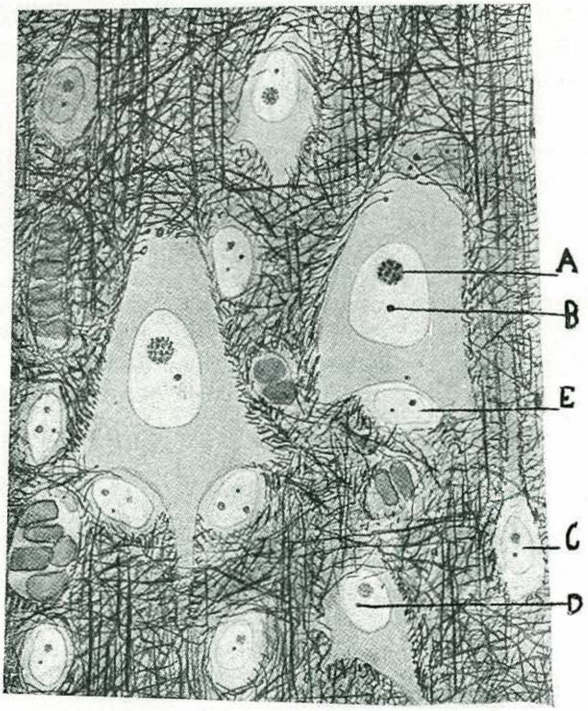

Fig. 47.—Terminal plexus about the pyramids. Cerebral cortex of the adult dog: *A*, nucleolus of a giant pyramid; *B*, basal neuroglial corpuscle.

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(1) CAJAL: Las células de cilindro-eje corto de la capa molecular del cerebro. *Rev. trim. Microgr.* Tom. II, 1897.

(2) If they were complete they would be so dense and so complex that they would be absolutely beyond analysis.

pictures of the terminal disposition of some axons and collaterals. This is due to its property of staining at times a few neurons or isolated fibers which come out vigorously on a clear and colorless background. But these revelations, to which I have devoted many years of incessant work (1), are obtained only in young animals or in small rodents 20 to 30 days old. However, it is possible to obtain some valuable evidence in the infant of one or two months.

Assuming that the organization encountered in the infant and in young animals does not undergo important transformations in the adult —which does not seem very probable— I shall mention here some synapses selected from many hundreds of thick and well stained sections taken from nearly all the regions of the cerebrum by various workers such as CAJAL, RETZIUS, MARTINOTTI, VAN GEHUCHTEN, CALLEJA, K. SCHAFFER, LORENTE DE NÓ and others. The following are the connections which seem to be most constant and least doubtful:

a) *Synapses between the extremely complex, diffuse nerve plexuses and the bodies and shafts of the cerebral pyramids.* — In this plexus of multiple origin the main components are the nerve collaterals emanating from the intra-griseal portion of pyramidal cell axons (2). It is obvious that nerve ramifications of many cells of short axon also participate, and possibly the terminal projections of the callosal and association fibers.

I referred particularly to this intricate plexus when I lamented the insurmountable difficulties facing an analysis of the cortical synapses. In figure 48 I present a schema of the course and probable connections of such nerve conductors based on the assumption that the main components are collaterals and dendrites. Note how these collaterals cross and enter into transversal or oblique contact with a great number of the dendritic shafts. It is probable that collaterals rest on the spines which cover the protoplasmic surfaces like down and it is likely that they also establish relationship with the cell bodies. Of course such synapses can only be effective between the neurons and the terminal

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(1) CAJAL: Studien uber die Hirnrinde des Menschen. Leipzig, 1906. Id. Sur la structure de l'écorce cérébrale de quelques mammifères. *La Cellule.* Tome VII, 1891.

(2) See the *Croonian lecture* in which I attempted to establish, at least hypothetically, the relationship of the collaterals of the pyramids with the shafts and bodies of other pyramids. (La fine structure des centres nerveux. *Proceed of the Roy. Soc. of London,* March, 1894.)

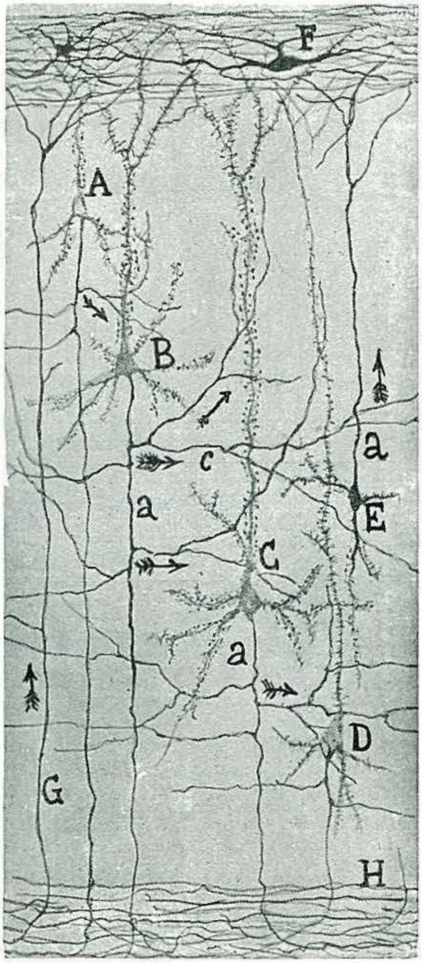

Fig. 48.—*A*, small pyramid; *B* and *C*, medium and giant pyramids respectively; *a*, axons, *b*, nervous collaterals which appear to cross and touch the dendrites and the trunks of the pyramids; *H*, white substance; *F*, special cells of the first layer of the cerebral cortex; *G*, fiber coming from the white substance. The arrows mark the supposed direction of the nervous current.

unmedullated portion of the mentioned collaterals, which are still enigmatic in the adult. This is the great disadvantage of using young animals in which the nerve collaterals have not as yet produced their myelin sheaths. It is for this reason that in a mouse or rabbit of 15 days it is impossible to distinguish the unmyelinated and branched terminal segment from the portion which will ultimately be enveloped by the myelin sheath. With regard to the terminal tips of these fibers all that could be said of them would proceed from conjectures more or less probable. Perhaps some of them terminate by means of varicosities upon cell bodies or dendrites located a great distance away. I have drawn such an arrangement at various times but it is so exceptional that it has almost always left me in doubt as to whether I am dealing with an artifact of impregnation or a true and constant reality. With respect to the adult animal, the Golgi method almost always fails tho show the course of the fine collaterals. The same can be said of the Cox method.

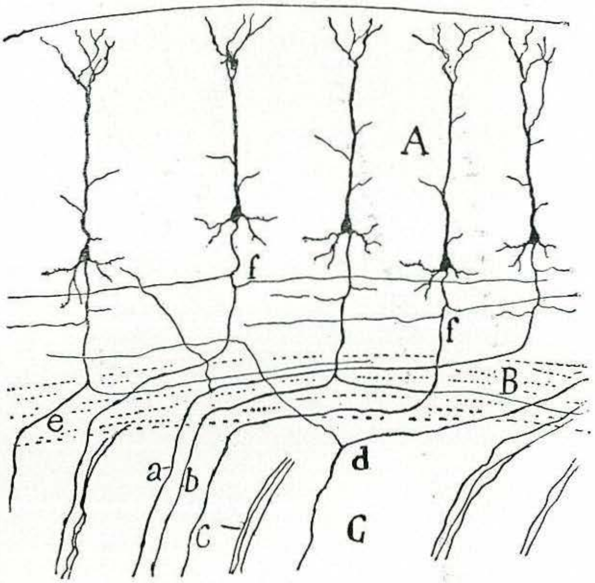

Fig. 48 bis.—Schematic representation of the enormous length of cerebral collaterals in the 20 day mouse: *A*, cortex; *B*, white substance; *C*, corpus striatum; *f*, long nervous collaterals. Note that some long collaterals *(c)*, in the white substance, are converted into association fibers.

The problem of cerebral axo-dendritic connections would be more understandable if the recent statements of Held published in a late paper could be confirmed (1). He considers the collateral spines as definite *Endfüssen* implanted on the dendrites (2). He even describes

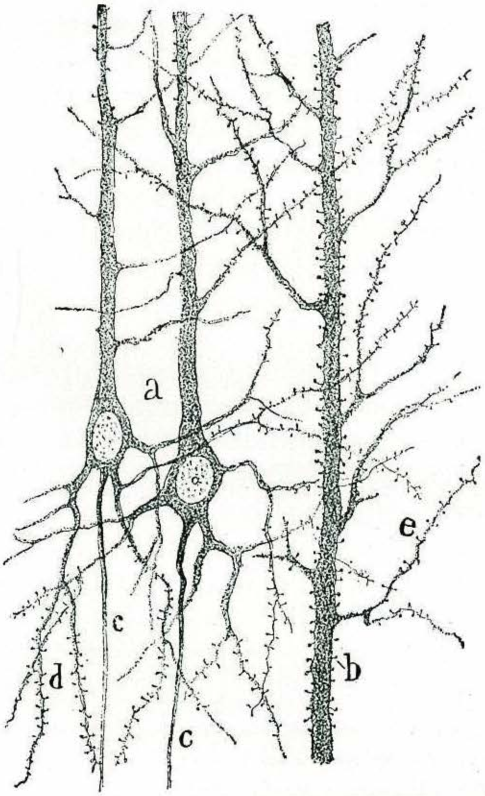

Fig. 49.—Cerebral pyramids of the nearly adult cat, stained by a variation of Ehrlich's methylene blue method: *b, c, d,* dendritic spines.

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(1) Held: Die Lehre von den Neuronen und vom Neurencytium und ihr heutiger Stand. *Fortschritte der naturwissenschaftl. Forschung.* N. F. Heft 8, 1929.

(2) Held attributed the discovery of the collateral spines of the dendrites to Golgi. I am sure that he must have seen them in his first preparations, but he probably considered them, as Kolliker did later, a silver chromate precipitate. At any rate, Golgi did not describe them in his early papers. (I have read all the volumes of his *Opera Omnia* as well as his early papers. See, for example, a paper published in 1883 in the *Archives Ital. de Biologie,* Tom. III, in which

some insterstitial nets which would put the pyramids in communication with themselves and with the terminal nerve fibers. I confess that, despite the great admiration which HELD's perspicacity inspires in me, I have not reached the point of accepting so unique an hypothesis. Without vainglory I can consider myself in this case a witness of the highest exeption because I have delicated more than 30 years to applying the method of GOLGI to the cerebrum and other nerve centers with a perseverance which could be equalled by others, but difficult to surpass. Now, I have never seen anastomoses between the spines and the nerve fibers despite the fact that I have devoted particular attention to them since 1888. Let us recall, in passing, that sometime ago Bethe considered the spines to be the point of departure of his interstitial *syncitium* (1).

I know well that in the realm of science that which is obstinately looked for is usually found; but when that which is not looked for establishes a frequent distribution and appears in all clearness it finally arouses the attention which was most distracted and most preoccupied with other problems. Neither GOLGI and his disciples, nor RETZIUS,

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he does not describe or draw the spines even in the Purkinje cells. Neither does FOREL mention or draw them in his communication *(Arch. f. Psychiatrie,* etc. Bd. XVIII, 1887) based particularly on the degeneration methods and the method of GUDDEN. Returning to GOLGI, the first time he mentioned and drew them was in his Nobel Prize lecture (December, 1906); but he attached no importance to them nor did he attribute the discovery to himself. On the other hand, I described and drew them in 1888 (cerebellum) and in 1891 (cerebrum) without counting that in 1896 I devoted a special paper to their study, staining them not only by the GOLGI method but also by a certain modification of the EHRLICH method. (CAJAL: Las espinas colaterales de las células del cerebro teñidas por el azul de metileno. *Rev. trim. microgr.* Tomo I, p. 151, 1896.) My analysis dispersed all uncertainty concerning the preexistence of such appendages. It is understandable then, how some authors, DEMOW, ODIER, STEPHANOWSKA, MANOUELIAN, etc., conceded them some role in nervous activity and supposed —without convincing proof— that by means of their retraction or swelling they might possibly intervene in the phenomena of sleep, wakefulness, narcosis, etc. For my part, I do not understand how HELD attributed his *Spitzenbesatz to* GOLGI and even to DEITERS, whose method of dissociation never clearly reveals such appendages. On the other hand, MEYER confirmed them in 1897 with the methylene blue method.

(1) See figure 26 in BETHE: Allgemeine Anatomie und Physiologie des Nervensystems, 1903.

Kölliker, van Gehuchten, P. Ramón, Calleja, Martinotti, Sala, K. Schaffer, Demoor, etc. —that is to say all those who in past or present times have explored the cerebrum, be it with silver chromate or Cox methods— not one of them have had the fortune of discovering the axo-dendritic nets of Held (1). But it is not enough to deny a supposed error —we ought to seek an explanation of its cause. I believe that Held has taken as interdendritic nervous nets the accidental unions at times produced by silver chromate deposited between the spines of proximate cells and the passing fibers. Perhaps the latter are unmyelinated collaterals. In any case such an accidental situation is exceptional.

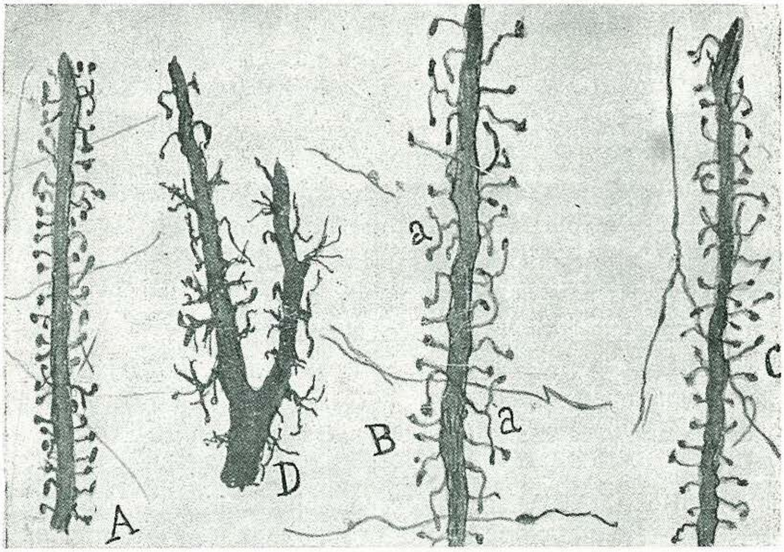

Fig. 50.—Types of collateral spines of cerebral pyramids: *A*, rabbit; *B*, child of two months; *C*, spines of a one month old cat; (visual region); *D*, a portion of a dendrite of a spinal motor neuron of the cat in a phase before the *Endfüssen* are formed.

Apart from these negative observations there exists a general and most powerful argument against the mentioned hypothesis of HELD, who incidentally, has not convinced BIELSCHOWSKY. I refer to the absolute impossibility of staining the dendritic spines of the cerebral pyramids and of the PURKINJE cells by the neurofibrillar methods. Nevertheless, they do frequently stain the finest nerve fibrils by means of which the collaterals of the cerebral pyramids terminate. How then are we going to mistake the base of a spine which is indifferent to colloidal silver and lacks a basilar thickening, with the authentic *Endfüssen* which are not only stainable by this reaction but also have a network or an interior ring? (1).

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(1) Lorente de Nó has made thousands of sections in the mouse, the monkey, the rabbit, and other mammals with the Cox and Golgi methods and never succeeded in detecting Held's cerebral syncytium. See his paper, rich in details on the dendrites and collaterals. Etudes sur le cerveau posterieur. *Trav. du Lab. de Rech. biol.* Tome XXIV, 1926.

I have discussed this problem several times but in order to illustrate it somewhat more I present in figure 50 several drawings taken from the shafts of young or adult cerebral pyramids. *A* represents the shaft of a pyramid from the visual region of a nearly mature rabbit. Note how short the spines are and how they begin very thin and end by a terminal bulb. Very few of them are bifurcated. In *C* the shaft of a pyramid of a one month old cat is shown. Here the arrangement described in *A* is confirmed; the spines appear a little bit longer and are frequently curved. In *B* (two month old child) I have drawn another shaft of a pyramid of the visual region. Here not only the greater length of the appendages but also the frequency with which they divide and change the direction of their secondary fibrils strike the attention. As a means of comparison I have included the dendrite of a motor cell of the spinal cord *(D,* one month old cat). Observe that the surface is bristling with irregular projections very seldom ending as bulbs. It is almost certain that this arrangement is transitory. Finally, I have drawn several cruciform or oblique nerve collaterals of the cerebrum. It is impossible to see any fusion of the latter with the spines.

b) *Terminations of centripetal fibers from the thalamus in the sensory cortex.*—In very complete impregnations the final ramifications of such fibers are best seen in the granular layer of the visual, acoustic and other sensory cortices where they generate a very dense and diffuse plexus. In figure 51, taken from my old sections, their intricate complexity can be appreciated. Within the plexuses granules and other cell bodies stand out as free spaces. Lorente de Nó has seen similar plexuses in the posterior cerebrum of the mouse.

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(1) It is certain that HELD, in one of his figures, reproducing a dendrite stained by his method for neurosomes, gives an enormous thickness to the base of the spines which is just the opposite of the delicateness of the origin of the genuine spines which he draws from silver chromate preparations. To appreciate this unusual contradiction, indicative that his drawings of the spines correspond to different things, compare figures 2 and 3 in the last monograph, so often cited by the Neurologist of Leipzig.

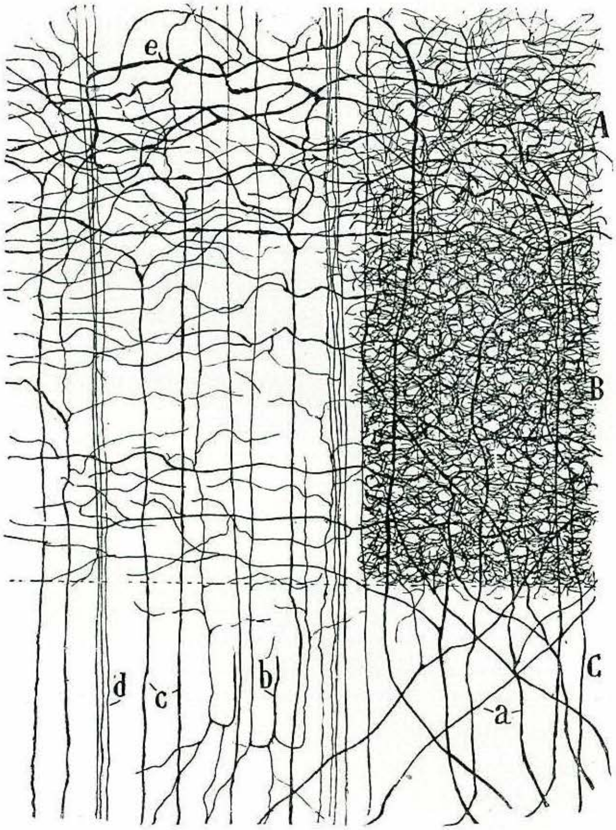

Fig. 51.—Nerve plexus of the fourth and fifth layers of the visual cortex of a 20 day old infant: *A*, fourth layer; *B*, fifth layer; *C*, sixth layer; *a*, optic fibers; *b*, axons of cells of the sixth layer; *e*, ascending axons of pyramid cells of the eighth layer; *d*, bundles of axons from the medium and large pyramids; *e*, arches of optic fibers with ascending collateral.

c) *Terminations of the neurons of short axon.*—In certain cerebral regions cells of short axon are encountered. This axon divides into a number of branches, each of which forms a nest for pyramids or some other types of neurons (1) (fig. 52).

In *Ammon's horn* and the *fascia dentata* the cells of short axons are the most typical, demonstrative and easy to study. There, in the vicinity of the bodies of granules and pyramids, I have been able to stain certain pyramidal or stellate cells with horizontal axons. From the latter emanates a multitude of terminal varicose filaments which intimately contact the bodies of granules *(fascia dentata)* or the pyramids *(Ammon's horn)*.

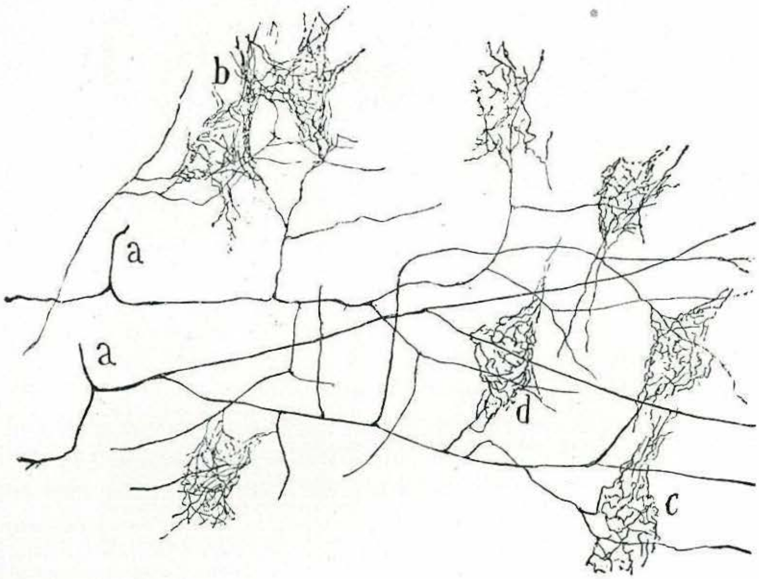

Fig. 52.—Pericellular arborizations of the medium and external giant pyramids of the motor cortex of a 25 day old child: *a*, axons dividing into long horizontal branches: *b*, *c*, *d*, pericellular baskets.

The nervous nests referred to above recall somewaht those of the cerebellum although they are poorer in terminal fibrils. They are best stained in the 15 to 30 day old rabbit with the GOLGI and COX methods. The slightly schematic figure accompaying the text dispenses me from entering into a detailed account. One interested in this matter can refer to my often mentioned complete work or to my old paper on *Ammon's horn* (1). (See figure 53, a, P.).

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(1) On the other hand, there are countless neurons of short axon which form diffuse and very extensive nervous arborizations.

d) *Axonal terminations of Martinotti neurons and similar types.*— The nerve arborizations generated by these axons embrace extensive areas of the first zone. In addition to furnishing collaterals to diverse cortical areas, the main stream of these nervous arborizations extends tangentially in the first layer, its branches coursing for great distances. In some instances I have observed condensations of the terminal fibers forming nests around the cells of short axon of the molecular layer, especially around the *Caja'lschen Zellen* of RETZIUS.

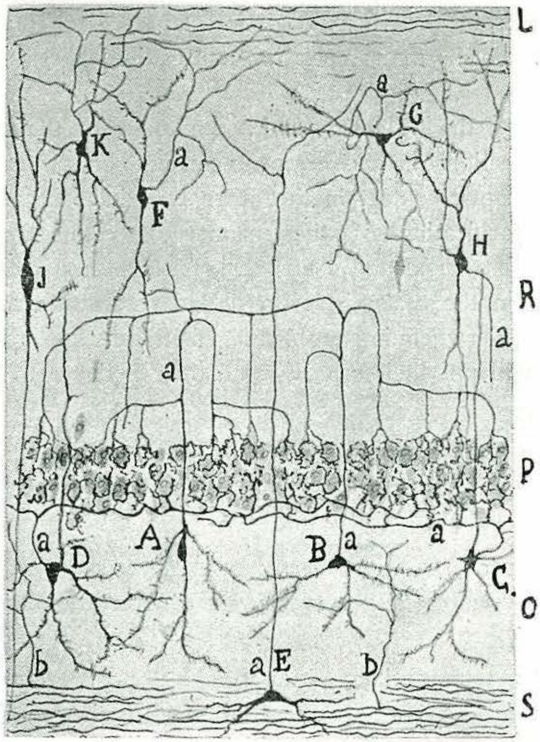

Fig. 53.—AMMON's horn of the rabbit: *A, B,* neurons in which the ascending axons decompose into arciform branches producing nests which envelope the bodies of the large pyramids *(P)*.

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(1) CAJAL: Estructura del *asta de Ammon y Fascia dentata. Annales de la Soc. Esp. de Hist. Nat.* Tomo XXII. 1893.

The same organization of diffuse arborizations presents itself to us in the ascending axons emanating from the deep cells of the cortex and in the descending ones recently described by Lorente de Nó. The latter never reach the white substance. In every respect the problem of the connections of the nervous factors of this layer is still very obscure. The great number of axons arriving as much from the white substance as from the various cortical layers also contributes to this problem. It may be that association and callosal fibers intermingle with these axons.

In summary, the little that is known today of the types of neuro-neuronal connections in the cerebral cortex, confirms in principle the manner of synaptic contact in the other encephalic regions. One thing is certain: clarification of the manner of connection between the innumerable centrifugal and centripetal, terminal and collateral branches emanating from the thalamic, callosal and association fibers constitutes at present an overwhelming problem. In it many generations of future neurologists will put their sagacity and their patience to the test.

CHAPTER X

11.—APPARENT OBJECTIONS TO THE NEURON THEORY BASED ON RECENT STUDIES OF THE SENSORY AND MOTOR END-ORGANS.

A. **Motor terminations and Boeke's periterminal network.** — The *motor end-plate* has been the field of extensive studies (1). In this structure the Gonheim method and the gold procedures (Loewit, Ranvier, Golgi, Ruffini, Cajal, etc.) have been employed with almost completely concordant results. Recently Boeke, using a modification of the Bielschowsky method, called attention to a very fine and pale framework which would extend throughout the nucleated territory of the motor end-plate. This reticulum, which would be present only occasionally, differs from the purely nervous branches of the motor endplate by its delicacy and by its limited affinity for the neurofibrillar reagents. The most interesting point about this discovery is the fact that on the one hand this pale and very subtle network would anastomose the terminal branches of the known nervous arborization, and on the other hand would be continuous with the dark bands of the striated muscle (1).

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(1) Boeke: *Anat Anz.* Bd. 35, 1910. Above all, see his work: Beiträge zur Kenntnis der motorischen Nervenendigungen. *Intern. Monatschr. f. Anat. u. Physiol* Bd. 28, 1911. Consult also the communication of Aoyagi (1912) and that of Boeke and Dusser de Barenne (1919) disciples of the Dutch scholar.

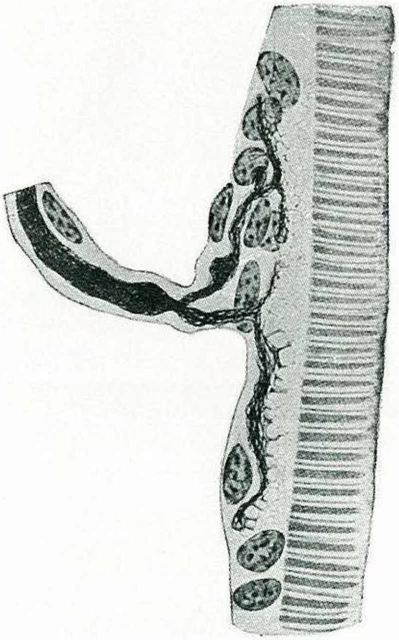

Fig. 54.—Motor endplate seen in profile (after BOEKE).

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(1) BOEKE's network is a rare phenomenon, so much so that it does not appear in most of the figures used by the Dutch scholar. That is why motor arborizations, identical or nearly identical to those I drew a long time ago (TELLO and other authors) are seen in BOEKE's paper. See, for example, the motor arborizations in his plates 17, 18, and 19. It is only in two or three figures of plate 20 that a pale, extrinsic network appears outside af the arborization *(Intern. Monatsch. fur Anat. u. Physiol.*. Bd. 28, 1911). The periterminal network is found more frequently in BOEKE's subsequent papers; for example in: Die Beziehungen der Nervenfasern zu den Bindegewebselemente

In figure 54 I present a drawing by Boeke which gives an idea of his conception of the plate's structure. After many attempts I have been able, with a special formula, to observe a reticular formation in the tongue of the rabbit. Although it appears thicker it recalls somewhat the *Netzwerk* of Boeke (1).

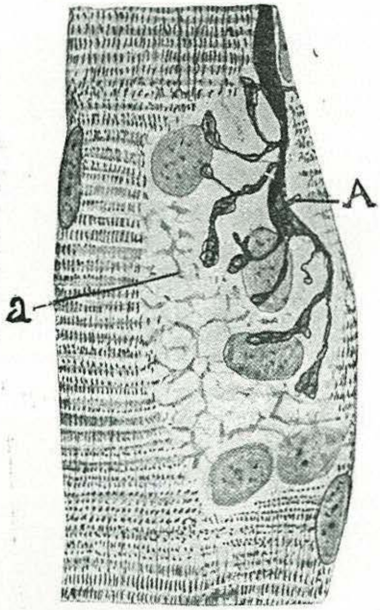

Fig. 55.—Rabbit tongue. Endplate seen almost in profile: *A*, thickening of the afferent axons; *a*, periterminal network of Boeke.

From these studies I have reached the conclusión that, besides the nuclei, the motor end-plate includes two essential structures: the

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und Tastzellen. Das periterminale Netwerk der motorischen und sensibeln Nervenendigungen, etc. *Zeitschr. f. mik-anat. Forschung,* Bd. IV, 1926. It will be profitable also to consult the works of Heringa (Untersuchungen uber den Bau and die Entwickelung des Peripheren Nervensystems, Amsterdam, 1920) and that of the Japanese worker, Iwanga (Studien über die motorischent Nervenendigungen. *Mitheil. u. allgem. Pathol. u. pathol. Anat.* Sendai (Japan) Bd. 11, 1925) and many others.

(1) Cajal: Quelques remarkes sur les plaques motrices de la langue des mammifères. *Trav. du Lab. de Rech. biol.* Tome XXIII, 1925.

subsarcolemmal ramifications of the nerve fibers and a pale network discovered by BOEKE (the periterminal network). But this reticulum, more or less granular in my sections (fig. 55), does not appear to interlace with the nervous arborization. On the other hand I believe it probable that it is continuous with the dark bands of the striated muscle, as I indicate in figure 55. At any rate, it is difficult to affirm with any certainty with which of them it enters into communication.

To sum up, and not to dwell more on this problem which I have discussed in a special paper, I believe that the plate possesses a characteristic reticular structure. This is also true of many other cell types such as epithelium (HEINDENHAIN), connective tissue cells (TELLO), ependymal cells (SERRA, CAJAL), cancer cells (DEL RÍO HORTEGA), neuroglia of the gray substance, etc. The reticulum is a vestige of the embryonic framework from which the striated material differentiated. I do not absolutely reject the idea that this framework of the plate might be an intermediary link between the contractile substance and the neurofibrils and neuroplasm of the terminal ramification.

Permit me to recall a fact ignored by the scholars. As early as 1904 I pointed out the neurofibrillar constitution of the nerve ramification of the plate (1). This was confirmed by TELLO (2) and other workers. Later BIELSCHOWSKY's school, employing ammonium silver oxide, also described them in all clearness.

Ribbon or membraniform terminations.—Do all the motor end-plates described by BOEKE belong to the same type? This seems doubtful to me. Besides the ordinary motor arborizations there also exist other relatively rare terminations with or without a periterminal network. I have observed them in young mice, cats, and rabbits, and in general in animals in the process of development. They consist, not of true ramifications, but rather of terminal expansions by means of flimsy, wide ribbons which appear in very fine network like a delicate mesh or veil. Perhaps the terminations described by BOEKE would belong to this type (figs. 19, 20 and 21 in his 1911 paper) (3). It is possible that IWANGA refers to them when he affirms that the *Netzwerk* could represent the marginal portion of undeveloped nerve branches which later on will be differentiated. As often happens, the membranous expansion only shows intensively stained axial neurofibrils which are continuous with the neurit, so that one could suffer the illusion of mistaking it for the diffuse periterminal network. But this illusion vanishes when we observe that the membrane exhibits well marked limits within the plate.

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(1) CAJAL: Contribución a la estructura de las placas motrices. *Trab. del Lab. de Invest. biol.* Tomo III, 1904.

(2) TELLO: Terminaciones nerviosas en los músculos estriados. *Trab. del Lab. de Invest. biol.* Tomo IV, 1905-1906.

(3) BOEKE: *Loc. cit. Zeitschr. f. mikr-anat. Forschung,* Bd. IV, 1926.

Let us recall that cones of growth possess the ability to develop elegant and very subtle veils when they encounter an obstacle. This is shown in the figures of my paper on nerve regeneration, especially in one representing a nerve membrane surrounding a fat cell (1).

Moreover, returning to BOEKE's periterminal networks in ordinary and adult plates, recent authors have expressed doubts about the neurofibrillar character of similar pale structures. Among them are CASTRO (2), who has examined sections from BOEKE's school, and Dr. WILKINSON (3), professor at Adelaida (Australia), who not only has

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(1) CAJAL: Demonstration photographique de quelques phenomènes de la régeneration des nerfs. *Travaux du Lab. de Rech. biol.* Tomo XXIV, 1926.

(2) F. DE CASTRO: Technique pour la coloration du systeme nerveux quand il est pourvu de ses etuis osseux, etc. *Travaux du Lab. de Rech. biol.* Tomo XXIII, 1925. This author writes: From our point of view this continuity of the neurofibrillar periterminals does not exist in the motor and sensory endings. It happens to be only an intimate adaption of surfaces without material or cement in the union between the protroplasm of the element innervated and that of the nervous termination. Under these conditions the impression is easily gained of a perfect continuity between the finest neurofibrillar trabeculae in the neuroplasm and the small argentophilic filaments disposed within the sarcoplasm or within the protoplasm of the sensory corpuscles.

(3) H. J. WILKINSON: The innervation of striated Muscle. Adelaide (Australie). 1921.

It is very significant that, according to this author, the periterminal networks, far from being stable as are the neurofibrils, fade away up to the point of disappearance.

According to this scholar, the true nervous termination of the motor plate is represented by the ordinary neurofibrillar arborizations, intensely stained, a uniquely permanent thing, which differs completely in color and appearance from BOEKE's network. Consequently, he doubts that the periterminal networks would conduct nervous impulses. It should be noted that this scholar, without knowledge of my work or that of CASTRO, agrees in principle with our conclusions, but in the defense of his opinions is even more radically and studied the preparations of BOEKE and AGDUHR but also has worked with these scholars. This investigator affirms that he has not clearly seen such periterminal networks. For the Australian author it is a case of some kind of separating partitions of intraplacular vacuoles which simulate a pale reticulum. Their presence is very inconstant. Neither have TELLO and his disciples reached the point of seeing them clearly with the GROSS and BIELSCHOWSKY methods.

B. **Terminations in smooth muscle fibers.** — Another argument advanced against the neuron theory is that of nerve terminations in the interior of the smooth muscle fibers described and drawn by BOEKE. The termination takes place by means of penetrating filaments stainable by the BIELSCHOWSKY method and ending by free, fine buttons. The figures of BOEKE were reproduced by HELD (1929) and many other authors, among them PH. STÖHR (1928). The button in question terminates sometimes within the protoplasm and other times within the nucleus (BOEKE). Somewhat similar terminations have bee described even in the interior of epithelial cells.

Assuming that such penetrating neurofibrils exist with certainty and that they do not constitute false appearances of external fibrils intimately adhering to the cell membrane, the fact is not incompatible with the neuron theory. For many years we all have known an authentic antecedent: the intracellular arborization lying in the motor end-plate and located beneath the sarcolemma. The examples cited by BOEKE would be, in principle, similar cases. I, myself, almost forty years ago described glandular intra-epithelial terminations (GOLGI preparations) (1), these having already been mentioned by PFLÜGER. Nevertheless, later on I became convinced that the fibers which I took to be

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strongly against the concept of the Dutch scholar. His categorical statement that the periterminal network, especially visible because of hematoxylin staining, disappears rapidly, is certainly surprising. An interesting discussion between BOEKE and WILKINSON concerning the structure of the motor-endplate can be read in the *Zeitschr. f. mikros-anat. Forschung,* Bd. 23, 1931. Also in the same volume is the work of HERINGA on the periterminal reticulum of BOEKE, with some interesting suggestions on the structure of the *sarcoplasma.* (Das BOEKE periterminale Netzwerk der quergestreiften. Musquelfasern, etc., p. 505.)

(1) CAJAL et SALA: Terminaciones de los nervios y tubos glandulares en el páncreas, etc. Barcelona, 1891.

intraprotoplasmic lie in the intercellular cement (1). But I repeat, the intraprotoplasmic penetration of a neurofibril clearly different in its appearance and color from the cellular framework does not constitute a proof against the neuron theory because the union of the two factors is lacking and their individuality is preserved.

Before me F. DE CASTRO expressed a similar opinion: "Even admitting", he says, "the invasion of the protoplasm of the smooth fibers as is supposed by BOEKE (1915-26), STÖHR (1926), LAWRENTJEW (1926) and of certain epithelial cells (BOEKE and HERINGA, 1920), it is none the less certain that the terminal fiber and the innervated protoplasm remain independent because their cytoplasms do not blend. Consequently, the nervous unity is maintained ... furthermore, the small mallet or terminal ring always possesses, besides the neurofibril, a tenuous sheath of neuroplasm. We are, therefore, in reality dealing with two independent systems intimately related". Such thoughts were formulated by DE CASTRO (2) because he saw or thought he saw the neurofibrils ending by means of buttons in the cells of the bone marrow during ossification.

C. **The periterminal network of Boeke in the sensory organs** (3).— BOEKE, firm in his intention of searching for his periterminal network in the different types of peripheral sense organs, succeeded in finding them in the corpuscles of GRANDRY (duck) and even in certain stratified epithelia.

In figure 56 BOEKE's drawing of the specific nervous disks of the corpuscles of GRANDY-MERKEL (duck) is reproduced. In the figure to the right the neurofibrils of the disk appear in frontal view, and in the one to the left the same disk appears in profile, situated between the two supporting elements. Though dimly seen, the continuity between the framework of the supporting cells and the neurofibrils of the nerve disk is none too distinct. The discovery of the DUTCH scholar has been welcomed with enthusiasm by HELD, who reproduced the figures of this author with belated delight.

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(1) CAJAL: For the terminations in smooth muscle fibers see my old paper. Los ganglios y plexos nerviosos del intestino de los mamíferos, etc. November 1893 (with 13 plates.)

(2) F. DE CASTRO: Quelques observations sur l'intervention du système nerveux autonome dans l'ossification, etc. *Travaux du Lab. de Rech. biol.* Tome XXVI, 1929.

(3) BOEKE: Die Beziehungen der Nervenfasern zu den Beindegewebselementen und Tastzellen. *Zeitsch. f. mikros-anat. Forschung,* Bd. IV, 1926. See also: Noch einmal das periterminale Netzwerk, etc. *Zeitsch. f. mikros-anat. Forschung,* Bd. VII, 1926. In addition to this, a network of fine filaments had already been seen by NAWIK in the cells of GRANDRY's corpuscles, but he did not affirm the continuity with the terminal disc. See: Zur Frage von dem Bau der Tastzellen in den GRANDRY'schen Korperchen. *Anat. Anz.* Bd. XXXVI. 1910.

As an indication of the interdependence of the nerve disk and the supporting cells, LAWRENTJEW affirms that the latter degenerate when the trigeminal nerve is sectioned (1). He believes that mitochondria of the cells undergo an early and very characteristic process of destruction and dislocation. In my opinion, such an observation lacks decisive value. When the functional bond between elements associated in the same activity is broken, it is logical that degenerative phenomena will follow. Concerning this problem let us recall the rapid metamorphosis of SCHWANN's cells in the peripheral end of sectioned nerves. In this case the degeneration could be explained, in principle, by the lack of

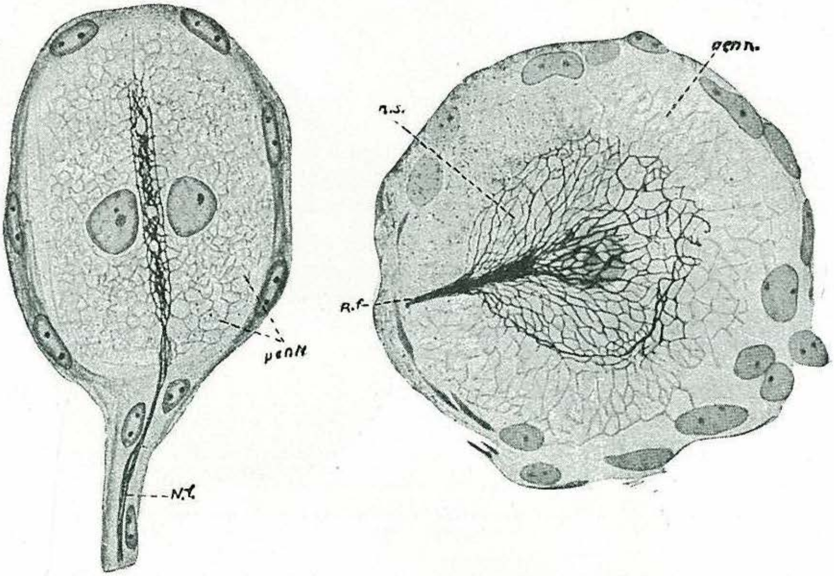

Fig. 56.—Nervous terminations in the organs of GRANDRY in the duck. At the right of the figure appears a section parallel to the plate: at the left, a normal section (after BOEKE).

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(1) LAWRENTJEW: Ueber das Chondriom der GRANDRY'schen Köperchen. *Zeitsch. f. mikros-anat. Forschung,* Bd. VI, 1926.

differentiation of the cells which, in the normal state, must receive from the trophic centers some dynamic excitant indispensable for the maintenance of their specific structure.

The periterminal protoplasmic network in the corpuscles of GRANDRY have also been the subject of anatomopathological experiments. Omitting other older experiments, I mention those of BOEKE (1), who after interrupting the innervation of these corpuscles, observed the penetration of sprouts into the nervous disks and the regeneration of the periterminal network. It should be recognized that his figures 26 and 27 (42 days after nerve section), where this network appears inside the regeneration of the periterminal *Netzwerk* in the supporting cells, are very impressive.

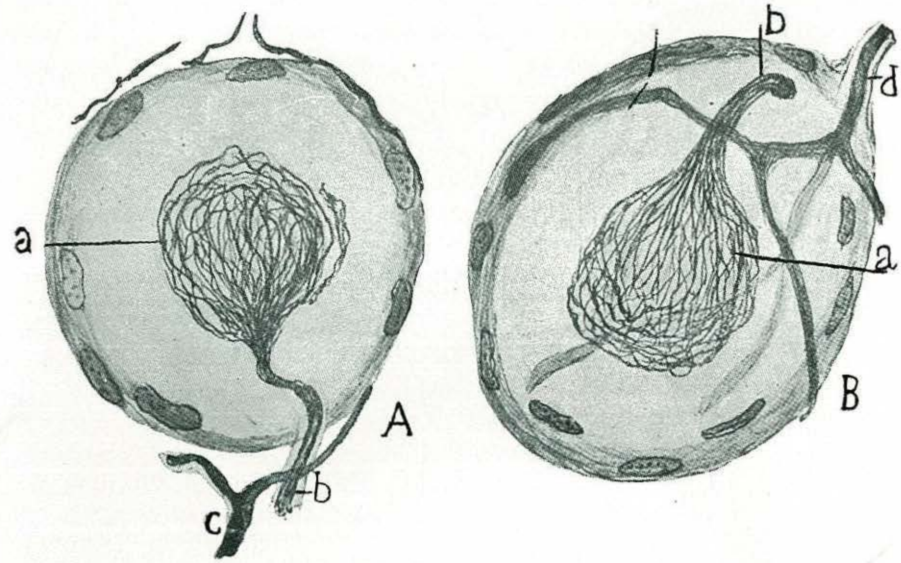

Fig. 57.—Structure of the nervous disc of the GRANDRY-MERKEL corpuscle. In addition, in the figure at the right there appears in the capsule what seems to be a terminal arborization, already known and described by the Russian school of DOGIEL: *a*, the neurofibrillar network of the disc; *d*, fiber going to the capsule.

On the contrary, the anatomopathological experiments conducted in our laboratory do not support BOEKE's thesis. The recent investigations of MARTÍNEZ PÉREZ (2), in which he has studied the regeneration of the corpuscles of GRANDRY and HERBST after section of the orbital branch of the trigeminal nerve, seem to deny the nervous nature of the mentioned periterminal network. The regeneration is very slow, beginning six months after the section and ending at the ninth month. I refer the reader to the interesting paper by TELLO's gifted disciple. Here it is only pertinent for us to recall the fact that, according to this author, the neurofibrils, regenerating and invading the old degenerated disk, do not pass beyond the limits of the disk; consequently, they do not assail the protoplasm of the supporting cells (1).

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(1) BOEKE: *Loc. cit. Zeitsch. f. mikros-anat. Forschung.* Bd. IV, 1926.

(2) MARTÍNEZ PÉREZ: Sur quelques faits intéressants touchant la régénération

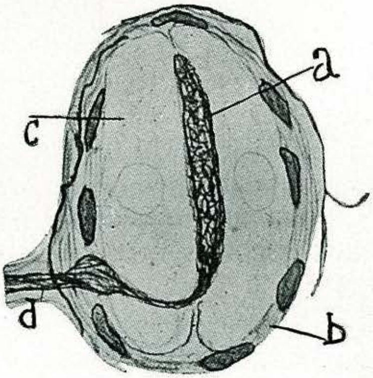

Fig. 58.—Organ of GRANDRY, sectioned transversly.

D. My recent investigations on the organs of Grandry and Herbst.—Recalling the success I attained years ago by applying the first formula of silver nitrate to this problem (sometime after DOGIEL (2), I

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experimentale dans les corpuscules de HERBST et de GRANDRY. *Trav. du lab. de Rech. biol.* Tome XXVIII, 1932.

(1) I consider the problem of the regeneration of the periterminal network still unresolved. This question demands more repeated new experiments and the use of reduced silver methods in addition to the formulas of GROSS and BIELSCHOWSKY for the examination of the lesions.

(2) DOGIEL: *Anat Anz.* Bd. XXV, 1904. In this work DOGIEL demonstrated for the first time the neurofibrils of the organs of GRANDRY and HERBST. See also his basic work done with EHRLICH's method. Die Nervenendigungen in den Tastkorperchen, etc. *Arch. f. Anat. u. Physiol. Anat. Abt.* 1891 and the

have attempted to stain the mentioned tactile organs. The fixative used is composed of silver nitrate (3 or 4 %) plus 15 to 25 cc. of alcohol. I have also had success with chloral hydrate fixation followed by ammoniated alcohol. The results obtained, in general, coincide with those of the BIELSCHOWSKY method and its modifications.

In figures 57 A and B, a, two intensively stained corpuscles of GRANDRY are presented in frontal view.

One should note that not a single neurofibril goes beyond the limit of the nerve disk. But perhaps sections parallel to the plate are not the most appropriate for observing the supossed periterminal network in the supporting cells. For this reason I have also studied anteroposterior longitudinal sections.

As can be seen in figure 58, a, the result has been the same. No filaments, destined for the supporting cells, emerge from the longitudinally cut disk in which the neurofibrils appear well impregnated. Reproduced in figure 58, d, is a well stained GRANDRY-MERKEL corpuscle in which can be observed the existence of the well known pericapsular nervous apparatus. It is independent of the axon generating the terminal disk (DOGIEL and his school).

During recent years the corpuscles of HERBST or small apparatus of PACINI in the bill of the duck have not earned an analysis as vehement and controversial as has the apparatus of GRANDRY. Although this question diverges somewhat from the problem with which we are dealing, I shall say something about them since they are impregnated intensively in my recent preparations.

Note in figure 59, in the terminal bulb of the nerve fiber, a free neurofibrillar network formed principally by loops, some of which protrude from the terminal axonal thickening. The interest of this observation, in my opinion, resides in the total absence of fibrils penetrating the granular central cylinder despite the intensity and delicacy of the impregnation.

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investigations of SZYMONOWICZ: *Arch f. mikros. Anat.* Bd. 48, 1896. I have even studied the corpuscles of GRANDRY with the silver nitrate method without, however, persisting on the subject which I believe was exhausted by DOGIEL. (See: Histologie du système nerveux. Tome I, p. 476, fig. 121.) In reality, nearly all we know about the neurofibrillar framework of the tactile discs we owe to the Russian histologist.

The organs of HERBST have acquired timely interst and theoretical importance thanks to the above mentioned recent work of MARTÍNEZ PÉREZ. This author has studied the traumatic regeneration of these organs and has proved that regeneration begins at two and a half months and reaches its highest peak in the eleventh month. But what is most curious and significant in these experiments is that the central nervous shaft is replaced by a complicated plexus of free branches which grow blindly in the thickness of the central granular mass without finally molding itself into a terminal mallet.

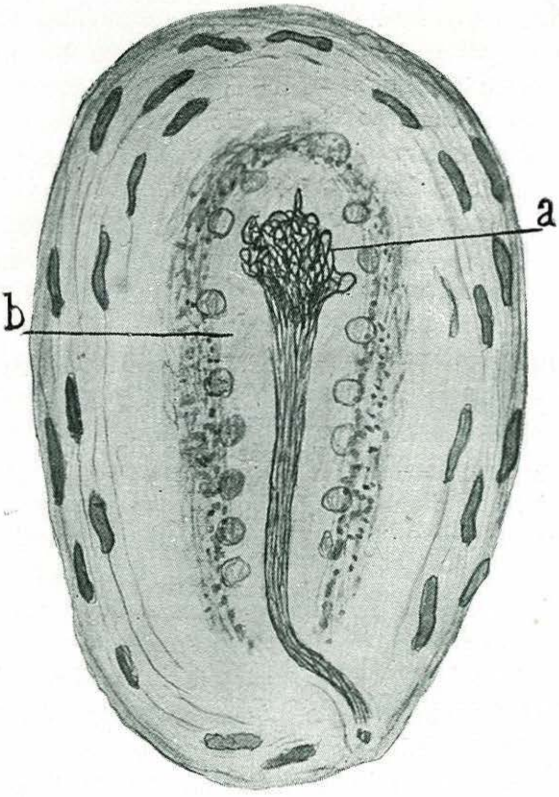

Fig. 59.—Corpuscle of HERBST from the bill of the adult duck (reduced silver nitrate method; fixed in nitrate-alcohol): *a*, complicated neurofibrillar plexus of the terminal bulb of the axon; *b*, pale protoplasm of the supporting cells free of neurofibrils.

Another interesting contribution of MARTÍNEZ in that all the new terminal branches advance freely without preëstablished conduits. This doctrine, which I have been upholding for more than 25 years, is opposed to the ensheathment theory *(Leitzellen* of HELD) among the adherents of which is BOEKE. In nerve regeneration, just as much as in embryonic development, the appearance of the *Leitzellen* is a late and never an initial phenomenon (1).

Let me make a general observation. In examining the figures in BOEKE's monograph, one is simply astonished to see the enormous contrast between the intensity of the staining in the neurofibrillar networks, in the nerve disks (and also in the motor end-plate) and in the periterminal networks. The staining is so pale in the latter that one can scarcely distinguish them. It is even more surprising in BOEKE's preparations that one never sees, or sees only with great difficulty, the sharp plasmatic limits which, in all peripheral end organs, separate the outline of the unmedullated terminal branches from the neighboring threadworks. These limits never cease to be seen in gold preparations (the LOEWIT method, for example) in those of DOGIEL where the ERLICH procedure is employed and in those made with silver nitrate (compare BOEKE's figures with mine, with TELLO's and with those of authors who have used the BIELSCHOWSKY method). Such uncertainty concerning the relationship of the periterminal reticulum to the neuroplasm constitutes one of the great inconveniences in the technique BOEKE employes by preference.

In conclusion, I do not deny the objective reality of the *periterminal reticulum* as I have stated above, but I oppose its strictly nervous nature and its supposed continuity with the neurofibrils. All of this in no way underestimates the physiological importance of the periterminal *Netzwerk*. It could belong exclusively to the innervated cell and represent an intermediary system designed to transmit the nerve impulse to the receptor organs (motor end-plates) or collectors (organ of GRANDRY). This nerve impulse could be received as well by the neuroplasm as by the neurofibrils, the exclusive conductive function of which is still hypothetical (2).

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(1) See mi book, unfortunately not well known, on the degeneration and regeneration of the nervous system.

(2) See amongst other works: Las células estrelladas de la capa molecular del cerebro y algunos hechos contrarios a la función exclusivamente conductriz

CHAPTER XI

12.—THE LATEST CONCEPTION OF HELD ON THE STRUCTURE OF THE GRAY SUBSTANCE.

Among the authors who have rejected most emphatically the neuron theory, mention should be made of HELD (1). His old theory of the structure of the gray substance is well known; all the free pericellular endings found during forty-five years of constant research on the nervous centers would send neurofibrils to the intracellular reticulum, the two being in continuity. Moreover, in the thickness of the gray substance would lie a syncitium composed of afferent nerve fibrils fused with the dendrites by means of the aforementioned *Endfüssen.* A good part of these hypotheses have been held for many years by HOLMGREN, WOLFF and STÖHR.

But the antineuronist attitude of HELD has been greatly accentuated by the ideas brought forth in his latest work. These are such that they baffle all of us who have preferred to use intense and precise selective methods and have devoted long years to the analysis of the problem of interneuronal connections.

The doctrinal point of departure of the Leipzig neurologist resides in the belief that the methods of metallic impregnation (GOLGI, COX, reduced silver, BIELSCHOWSKY and others) stain only a part of the nervous structures, in which case an essential factor of the gray substance ordinarily escapes their selective action, particularly at the level of the molecular layer. This essential factor is a *diffuse protoplasmic syncitium,* which from various aspects, recalls that described by GERLACH, APATHY, NISSL and BETHE, although there are important differences.

HELD mentioned the molecular layer of the cerebellum as an example

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de las neurofibrillas. *Trab. del Lab. de Invest. biol.* Tome IV, 1905. See also: Das Neurofibrillennetz der Retina. *Int. Monatsch. f. Anat. u. Physiol.* Bd. 21, 1904. (In the retina a whole system of very important conducting cells (the bipolar cells) lack visible neurofibrils; on the other hand, neurofibrils are extremely abundant in the horizontal neurons. the collaboration of which in the transmission of nerve impulses is rather doubtful, etc.)

(1) HELD: Die Lehre von den Neuronen und vom Neuronencytium und ihr heutiger Stand. *Fortschr. der. naturwiss. Forschung; Neue Folge,* H. 8, 1929.

of his new concept. He describes a pale fundamental network, unstainable except by the non-selective methods and somewhat similar to the networks described in Gerlach's time. This reticulum *(Grundnetz)* is of a protoplasmic nature and should be considered as a new structural factor. Neurofibrils emerging not only from the tips but also from the sides of the dendrites participate in this *Grundnetz*. As for the neurites, they also would take part in the formation of the fundamental reticulum, the latter being in continuity with Golgi's pericellular network. This *syncitium* would exist in the cerebrum as well as in the cerebellum, extending to the white substance where Bethe described it, but above all, it would be found in the molecular layer as above mentioned.

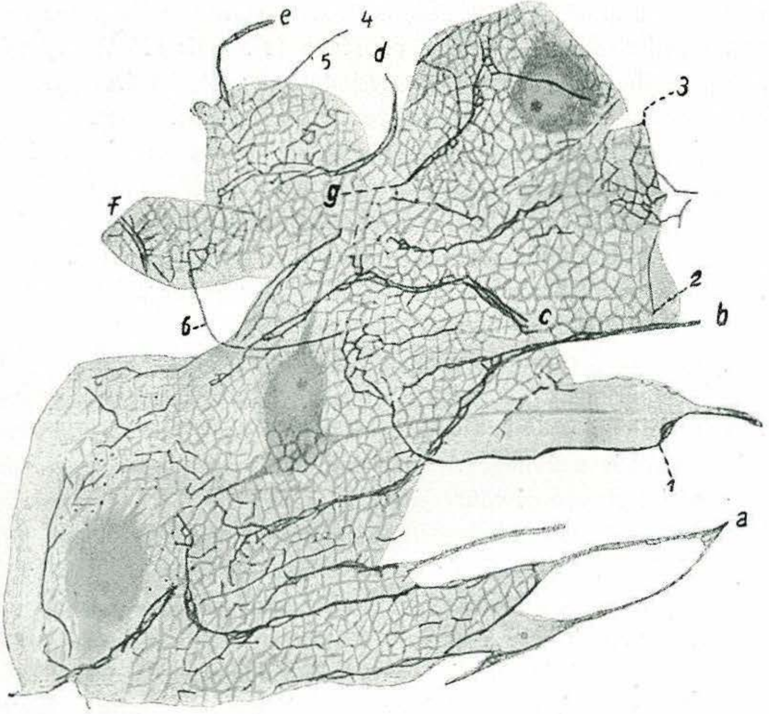

Fig. 60.—Piece of the molecular layer of the cerebellum, after Held. One sees Purkinje dendrites in which the neurofibrils are lost in a pale interstitial reticulum: *b*, Purkinje dendrites; *1, 2, 3, 4*, neurites; *c*, neurofibrils emptying into the *Grundnetz*.

There would not be, therefore, true neurofibrillar terminations in the nerve centers. But what is more surprising in Held's doctrine is the participation of the neuroglial in this syncitium (neuroglial *syncitium* of Hardesty, Fieandt and others), which would be joined to the fundamental reticulum. Thus is destroyed the doctrine of the independence of the nervous and neuroglial cells, defended, since the time of Deiters, Ranvier, Max Schultze and Golgi, by all of us who apparently accepted it with delightful naivete.

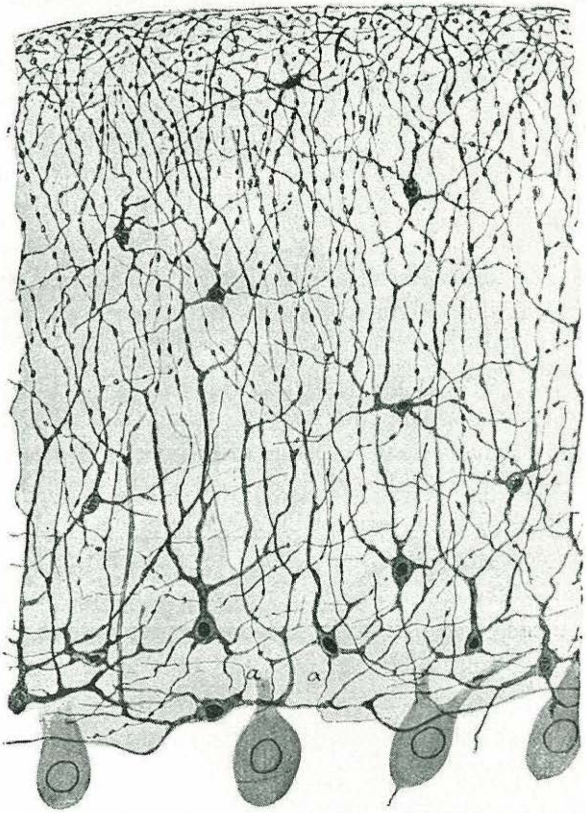

Fig. 61.—Dendritic branches of the basket cells stained by the Ehrlich method. Molecular layer of the cat.

Unfortunately, Held's figures and descriptions are not convincing, as Bielschowsky pointed out. This occurs in his figures 18 and 19 (fig. 60), which represent a part of the molecular layer of the cerebellum.

A few dendrites appear intensively stained by a silver method and interlaced with the fundamental reticulum. In this field there are a few neurites from an unknown origin (fig. 60, 1, 2, 5, etc.). It is my opinion that the very few neurites figured, probably belong to the basket cells. As to the extremely rich arborizations of the PURKINJE cells, the transversal fibers and the climbing fibers, etc., they are striking by their absence. Among the neuroglial elements one does not observe the radial fibers (forked cells, well drawn by GOLGI and by all those after him who worked with his methods), the neuroglial corpuscles of FAÑANAS, stainable by gold and confirmed by SOMOZA, or finally, the numerous other factors that go to make up the mentioned molecular layer (cells of Río-HORTEGA, etc.).

As a proof of the enormously complicated structure of the molecular layer of the cerebellum, figure 61 shows the dendritic branching of the basket cells stained by the ERHLICH method. To complete this impression of complexity the reader should consult my papers on the cerebellum or, if not available, figures 34, 35 and 62 of the present work.

One can guess that HELD, in order to present in a clear manner the *Grundnetz,* has omitted almost all the nervous and neuroglial structures of the cerebellum. It was permissable to defend the reticular hypothesis regarding the cerebrum and spinal cord in former times (the days of GERLACH and DEITERS). But after the advent of the GOLGI and WEIGERT methods, BIELSCHOWSKY's metallic preparations and those of the Italian and Spanish schools, etc., the continued acceptance of an interstitial, continuous reticulum with nervous function, resistant to the intensively selective methods used for glia and nerve fibers (although it may be masked under the equivocal designation of *protoplasmic* network), seems not only a strange anachronism but a hazardous and unacceptable hypothesis as well. Yet this *Grundnetz* could be understood if it is considered as a non-nervous factor, that is, one without a power of conductivity. But to regard it as an intermediary bridge capable of transmitting diffusely the specific nerve impulse and, moreover, as being in continuity with the neuroglial expansions, is an almost metaphysical concept which transcends my credulity. As with most synthetic speculations, this theory is faced with all the inconveniences and contradictions of conciliatory doctrines which, in an attempt to satisfy everybody, convince nobody or at best very few.

With all due respect to a scholar like HELD, who during his youth enriched neurology with important findings by using the ordinary methods, his own procedures and above all, the silver technique, permit me to make some critical observations.

1. A praiseworthy although very dangerous preoccupation, already thought of by NISSL, runs through the hypothesis of HELD: that of filling, by all possible means, the clear or molecular zones of the gray substance with new nervous structures. This analytical obsession of filling in the unknown ground of the cerebellar and cerebral cortices with simple and schematic structures is the same one I also suffered fifty years ago when I stained sections with carmine and hematoxylin, that is, when the marvelous analytic powers of the GOLGI and EHRLICH methods were as yet unknown. But now that the silver procedures have demonstrated that the molecular layers are organized in plexuses, totally different thoughts absorb me. Thanks to the action of silver chromate, we have filled in completely the mentioned *terra ignota* with countless unmedullated nervous arborizations and infinite dendritic ramifications, plus an uncalcuable number of neuroglial fibers and cells. Consequently, I ask, where would space be found for a new structural factor so diffuse and rich, when it seems that the available space in the molecular layers is exhausted by the known neuroglial and nervous strutures located there (plexiform layer of retina, cerebrum and cerebellum, ROLANDO's substance, etc.)? In principle, I don't deny the existence of some intercellular substance but I conceive it as a fluid or semi-fluid capable of being coagulated by the reagents. This substance, under the action of the non-selective methods, will appear somewhat like a system of very tenuous alveoli. But since this system is not stained by the GOLGI or the neurofibrillar methods, I am inclined to think that it is not of a nervous nature.

2. HELD seems to forget the importance of artefacts due to fixatives or, at least, he does not give them due importance. Always when alcohol, formaldehyde and other formulas of fixation are used, followed by staining with anilines, hematoxyline or carmine, two important phenomena appear: the formation of interstitial vacuoles of variable size and above all, the aproximation or something like a fusion of the filaments and limiting membranes of the nervous and neuroglial expansions. Probably this is a case of an agglutination which seems

absent in metallic preparations and is masked because the nervous and neuroglial structures, interconnected throughout the mentioned membranes, stand out intensively, leaving the background unstained and as if it did not exist.

Similar pictures have been observed by other authors, especially by ACHÚCARRO (1). This author, using formol-uranium, has seen a pale interstitial network which is not very much like those described by HARDESTY, HELD, FIEANDT and ALZHEIMER. A detail concerning this interstitial network is important. It does not appear in continuity either with GOLGI's pericellular reticulum or with the expansions of the neuroglial cells. These facts incline one to consider it an artefact.

3. Instead of giving incomplete pictures, the metallic impregnation methods are infinitely richer in details than those of other methods, not excepting the neurofibrillar procedures. Nevertheless, none of them constitutes as yet an ideal method. Despite all this, and notwithstanding its limitations, the colloidal silver has confirmed in great part, the incomplete details given by silver chromate and, in addition, it has supplied important details concerning the structure of the gray substance.

4. Note that in HELD's schematic figure (fig. 60), the secondary and tertiary dendrites of the PURKINJE arborizations, which are faithfully reproduced in figure 62, are not represented. If we judge by the accompanying schema, surely HELD, using the BIELSCHOWSKY method, has discovered only the neurofibrils of the primary heavy branches. From the borders of these branches very subtle threads would emerge as jets, and lose themselves in the *Grundnetz* or background network.

Besides very expressive GOLGI preparations, I have neurofibrillar

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(1) ACHÚCARRO: Notas sobre la estructura y funciones de la neuroglia, etc. *Trab. del Lab. de Invest. biol.* Tome XI, 1913. Figure 3.

See CAJAL: Studien über die Hirnrinde des Menschen. Translated by DOCTOR BRESLER. J. AMBROSUIS BARTH, Leipzig, 1900. In this work is seen the molecular layer, particularly the first layer of the cerebral cortex, made up of a very dense triple plexus of nervous ramifications, dendrites and neuroglial appendages. Likewise it is the same in the molecular layer of the cerebellum where, in addition to the extremely dense system of parallel fibers, there are also the Purkinje cell dendrites, the climbing fibers and the axons and the very tufted dendritic branches of the basket cells (see figure 61). To this there must also be added the radial fibers and their collateral excrescences, the microglial and the cells of FAÑANAS.

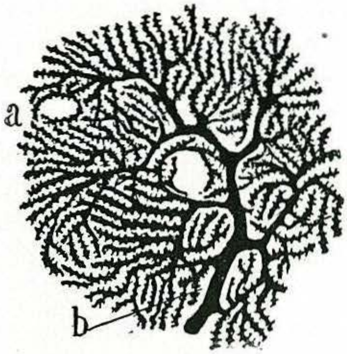

Fig. 62.—Tertiary terminal branches of a PURKINJE cell of man: *a*, cavity for capillaries; *b*, terminal branches copied with all possible exactness. (GOLGI method).

impregnations of the PURKINJE dendritic system in man (1907), much more complete than those of HELD (fig. 63). Now contrary to the opinion of the Leipzig histologist, it is impossible to observe such a neurofibrillar dispersion in the *Grundnetz* background. There does not appear the least

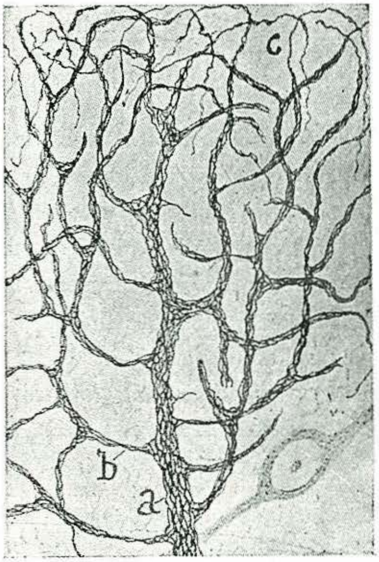

Fig. 63.—Neurofibrils from a portion of the dendritic branches of a PURKINJE cell of man; *a*, primary trunk; *b*, secondary branch; *c*, tertiary branch. Most of the latter are still not impregnated (reduced silver nitrate.)

vestige of the pale reticulum in question. Actually, to obtain these preparations I used as a fixative, not formaldehyde, but 97 % alcohol which has the advantage of fixing well without diminishing the affinity of the neurofibrils for colloidal silver.

5. The above mentioned protoplasmic interstitial reticulum of HELD lacks affinity for BIELSCHOWSKY's colloidal silver, as well as for silver chromate and methylene blue (ERHLICH's method). These negative properties do not, therefore, favor the admission of a nervous function for this network.

6. Neither are HELD's figures concerning the continuity of the *Grundnetz* and the neurofibrils of the neurites completely convincing. Examing carefully his figure 17 (my fig. 60), one receives the impression that these emerging neurofibrils are not fused with the protoplasmic network *(Grundnetz)* as they enter its territory, but rather that they are superimposed on it and stand out sharply because of intense impregnation (1).

7. The conception of HELD presents insurmountable difficulties to the doctrine of physiological localizations, and for any plausible interpretation of the propagation of the nerve impulse through the gray substance because any attempt to determine its direction would be an impossible endeavor.

8. The concept of a syncitium in the gray substance (FIEANDT, HARDESTY, HELD, etc.) and its continuity with the *Grundnetz* is no less troublesome. This concept rejects the generally accepted idea of the autonomous morphology of the neuroglia, proved by methods of strong selectivity and even by the dissociation methods used by DEITTERS and RANVIER. Moreover, because of its supposed relationship with the *Grundnetz,* it would force us to attribute to the neuroglia, an important role in the process of nervous activity.

For all these considerations and many others that I omit to avoid annoying repetition I consider the very new conceptions of HELD concerning the fine structure of the gray substance unacceptable. He attempted to reconcile in principle the contradictory facts and hypotheses of NISSL, BETHE, FIEANDT and other illustrious masters of German neurology, including BOEKE, whose *ultraterminal networks* recall somewhat the doubtful *Grundnetz* of the cerebellum and cerebrum. This laudabel and engaging desire, as well as the gentleness and courage of his attempt, are points in his favor.

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(1) I think it probably that the neurofibrils, which according to HELD (figure 60) appear detached from the dendritic trunks, do not course freely throughout the background network but that they are found on the unstained neuroplasm of secondary branches of Purkinje cells. In my preparations such filaments always appear enveloped in a transparent but well limited material.

PART TWO

CHAPTER XII

I.—NEUROGENETIC UNITY AS A PROOF OF THE NEURON THEORY. FREE GROWTH OF THE AXONS IN TISSUE CULTURE. ERROR OF THE SUPPOSED INITIAL ENSHEATHMENT IN THE COURSE OF THE EMBRYONIC AXONS. FREE GROWTH OF THE NEWLY-FORMED FIBERS IN THE SCAR OF THE SECTIONED NERVE.

A. **Neurogenetic unity of the neurons and axons.** — As HIS presumed and LENHOSSÉK, RETZIUS and I have demonstrated with irrefutable observations, every neuron results from the evolution of a *germinal cell* of the primitive *medullary tube,* passing through the following phases: (a) *bipolar cell;* (b) *neuroblast* of HIS, a pyriform cell provided with a short axon terminating in a cone of growth (CAJAL, LENHOSSÉX, etc.); (c) a small neuron with rudimentary dendrites; and finally; (d) a stellate cell having two kinds of differentiated and ramified projections: the *neurite* or axon and the *dendrites,* both ending freely. This neurogenetic cycle is common to all nerve cells with some variations, especially in the cerebellum, retina and dorsal root ganglia (1).

Fortunately, on this point, the opinions of all who have studied the evolution of the neuron in modern times, are in accordance, including even the preoccupied and fastidious HELD himself. To the reader interested in neurogenesis and desiring broader information, I advise him to consult the basic papers of HIS and the modern ones by HELD, HARRISON, LUGARO, MARINESCO, AGDUHR, etc., based for the most part on the use of neurofibrillar methods. In this article, because of its synthetic character, I shall mention only the opinions relating to the neuron theory. Figures 64 and 65 demonstrate some typical phases in the development of the spinal cord cells.

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(1) A collection of my monographs on neurogenesis have recently been published in a volume under the title: Etudes sur la neurogenese de quelques vertebres. Madrid, 1929. The investigations of HELD, which appeared at different times are found developed and profusely illustrated in his classic book: Die Entwicklung des Nervengewebes. Leipzig, 1909.

It is worthy of note that once the chain theory of axonal evolution and regeneration was abandoned, disagreements involved only secondary questions.

At first glance it could be supposed that between HELD and me there exist radical differences of interpretation. Nevertheless, nearly all can be reduced to a chronological question. For example, HELD, together with HIS, VON LENHOSSÉK, KÖLLIKER, RETZIUS, LUGARO, HARRISON, TELLO, LEVI, etc., admits the unity and independence of embryonic neurons, and maintains, as do I (1), that the primordial neurites terminate by means of a *cone of growth,* and finally, that the axis-cylinders constituting the young nerves are an uninterrupted continuation of these neurites. I agree with this opinion and only differ in this detail: in my opinion the *exploring fibers* emerging from the medullary tube and arising from the neuroblasts would course at first, that is, when they are few in number and isolated, through the interstices of the mesoderm; whereas later on, once they have been gathered into loose fascicles, making up the rudimentary roots, there would appear the sheath cells, the origin of which is enigmatic (the *lemmoblast* of VON LENHOSSÉK, the *Leitzellen* and *plasmoderma* of HELD). Besides the fact that the *Leitzellen* are not indispensable is proved by many experiments, for example, those of HARRISON, who extirpated the neural crests of tadpoles, the source of the primitive SCHWAN cells, and without their coöperation the axons differentiated, grew, and arrived at their destination.

According to the neurologist of Leipzig, immediately after the cones of growth would emerge from the spinal cord they would insinuate themselves into the thickness of certain cells *(Leitzellen)* which would serve as guides. Thanks to them the cones would grow and reach their point of destination. Let us note that if HELD's interpretation were exact, it would not substantially affect the neuron theory since the protoplasm of such adventitial cells is neither continuous with the neurofibrils nor possibly with the neuroplasm of the embryonic axons.

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(1) CAJAL: Genèse des fibres nerveuses de l'embryon, etc. *Trab. del Lab. de Invest. biol.* Tome IV, 1906. Idem Estudios sobre la degeneración y regeneración del sistema nervioso, 2 vols., 1912-14. There is an English translation by DOCTOR MAY: Degeneration and Regeneration of the Nervous System. 2 vols. Oxford University Press, London, 1928. The works of TELLO, little known, have been published in *Trav. de Lab. de Rech. biol.* One above all merits special mention: Les différenciations neuronales dans l'embryon du poulet pendant les premiers jours de l'incubation. Tome XXI, 1923.

I would be very happy to accept HELD's thesis if the following facts would not detract from its credibility:

1. The exploring axon or the axon just emerging from the spinal cord can grow freely through the mesodermic interstices, but most often it is supported during its exodus towards the periphery by embryonic fibroblasts over which it glides by virtue of a phenomenon of stereotropism. The latter, well studied by LOEB and HARRISON, has been confirmed by me many times in the embryo as well as in nerve sprouts of scars (the regeneration process of sectioned nerves). Given this curious and well known property and the variable forms of the *Leitzellen* and *plasmoderma*, it is not surprising that in a transversal or longitudinal section of the growing roots it was possible to deduce that a free neurite, intimately superimposed on the mentioned elements which are more or less channeled or tortuous, was located in the thickness of these cells. It is

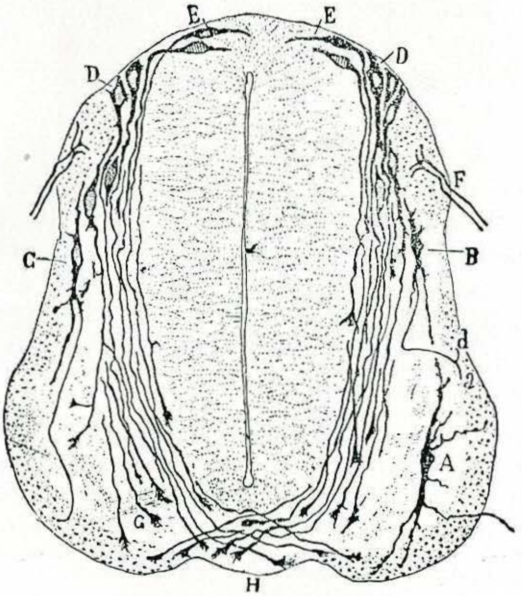

Fig. 64.—Spinal cord of a 4 day chick embryo: *A*, motor neuron; *c*, growth cones proceeding in the direction of the raphé. (GOLGI method.)

possible to see in figure 65, A, D, how the cones of growth of these primitive neurites insinuate themselves between the mesodermic cells without penetrating their interior.

2. The culture experiments of HARRISON's school, confirmed by BURROW (1910), LEVI (1911), MARINESCO and, in our group, by SANZ, demonstrate the aptitude of the cones of the embryonic fibers to grow freely through artificial plasmas. They apply themselves indiscriminately to fibrin filaments to proliferated mesodermal cells and to other nerve fibers (CAJAL's reciprocal stereotropism), sometimes producing the appearance of *interneuronal* anastomoses as LEVI has well pointed out (1).

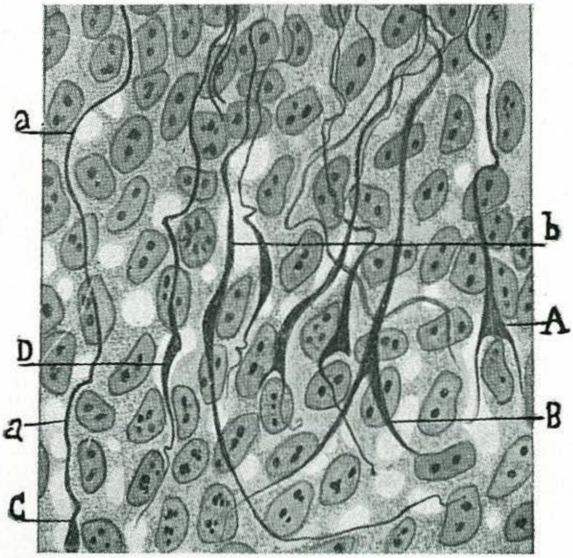

Fig. 65.—Growth cones arriving in the perimedullary mesodermic tissue: *A*, *B*, *D*, note how they move freely between the cells. Background stained with hematoxylin.

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(1) Nevertheless, LEVI, in some preparations of old cultures of nervous tissue admits anastomoses between axons of different neurons (see figures 549 and 550 in his Trattato di Istologia (1927). MOSSA (1929) and OLIVO (1927) and others have likewise observed atypical anastomoses in tissue culture.

Supposing that the Italian scholar has not mistaken intimate juxtaposition of fibers (reciprocal stereotropism) for anastomoses, it should be remembered that transplantation of fragments of the spinal cord to artificial media creates abnormal conditions of growth and evolution, capable of disturbing the tendencies

Figure 66, a, shows cones of growth in the optic lobe of a chicken embryo coursing through the blood plasma of this animal without relying on the mesodermal cells.

3. I have seen similar free cones of growth in the ependyma, and other organic cavities abundant in plasma (CAJAL, LORENTE DE NÓ, TSCHERNJACKIWSKY (1928), etc.).

4. It seems unlikely, as LUGARO noted, that the primordial nerve fibers would be incapable of proceeding freely in the mesoderm, when we know that during the development of the nervous centers they grow in the latter with extreme rapidity across the embryonic gray framework and that they join with other axons and form nervous pathways and robust nerves without the aid of any guiding cell.

5. Finally, I believe, *a priori*, it highly impossible that the exploring cones of a soft consistency, as seems to be demonstrated by their bending and recoiling from the very first as they encounter the basal or primary projections of the perimedullar mesoderm would be capable of perforating series of *Leitzellen* or mesodermic cells which are unchanneled and probably as soft and viscous as the cones. But I shall not dwell upon this point which I have discussed extensively in previous books and papers. Here I shall limit myself to noting that HELD's hypothesis of ensheathment (embryonic development) leaves the problem of orientation intact. In reality, it shifts the difficulty without resolving it. If this concept were admitted it would follow that the *Leitzellen* would be disposed in the mesoderm beforehand in oriented chains by some kind of miracle. Such an explanation (which exhibits teleology and even predestination, both incompatible with the spirit of modern natural philosophy) is much more difficult to comprehend than that of automatic regulation of the free course of the cones by the action of chemotactic substances or electric phenomena (hypothesis of SFRASSER and similar ones) (1).

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of the sprouts. LEVI, himself, is in complete agreement with this opinion and he affirms that it is rather debatable whether the facts observed in tissue culture can be applied without reservations to normal neurogenesis. Let us note that LEVI, a converted neuronist, has confirmed the influence of the stereotropism of LOEB and HARRISON so evident in the scar of early regeneration of sectioned nerves.

(1) CAJAL: Nouvelles observations sur les neuroblastes, etc. *Anat. Anzeiger,* Bd. XXXII, 1908.—Idem: *Loc. cit.* La dégeneration et la régeneration, etc. Tome I, 1913.

If the intercellular neurofibrillar anastomoses pointed out by HELD in the GASSERIAN ganglion of the duck and the bridges and fusions described by BIELSCHOWSKY (1928) in human ganglia (embryo of 1.6 cm.) were constant conditions and could be easily proved, they would be more

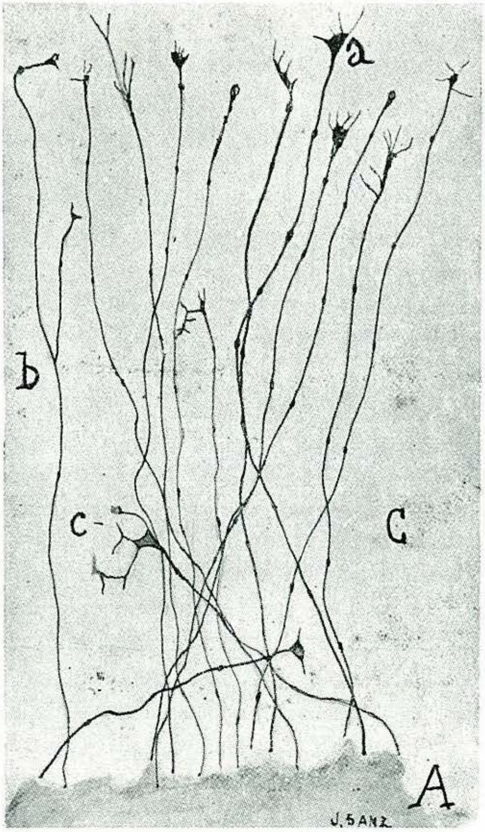

Fig. 66.—Optic lobe of an 8 day embryo, maintained in an artificial medium for 24 hours. Impregnated by BIELSCHOWSKY's method: *C*, plasma or culture medium; *a*, our cones of growth; *b*, bifurcation of an axon. (Prepared and drawn by SANZ.)

serious as objections to the neuron theory. Unfortunately, such anastomoses bring to mind those described in the spinal cord a long time ago by the older anatomists (recall FRAGNITO, JORIS, SEDWICH, etc., who worked with special methods). They would be so exceptional that in several hundreds of embryonic preparations of mouse, chicken, various birds, etc., made by me and TELLO, we have never been able to observe them clearly. It is true that various cases, especially when pure pyridine is used as a fixative (1), some sensory corpuscles touch each other and unite as compact masses, but in them a clearly evident picture of neurofibrillar continuity is never recognized. In summary, these fusion phenomena seem to me to be artifacts resulting from the disturbing action of the fixatives. (I mention in passing that there exists no perfect fixative.) Even if them were true, what would such sporadic and ephemeral arrangements represent along side of thousands and thousands of sensory corpuscles which show their independence clearly in perfect preparations, intensively stained, during all phases of their development? Moreover, these supposed anastomoses are absolutely absent in the adult.

B. **The theory of autoregeneration and ensheathment in the regrowth of sectioned nerves.**—Today the arguments brought forth from the theory of nerve regeneration have lost all their controversial value. This is not at all surprising. The theory of *autoregeneration,* that is to say, the process of discontinuous generation of the axons at the expense of the cells of SCHWANN at the terminal end and without the collaboration of the trophic centers or the neurons of origin, appeared at a time when there were no adequate methods for impregnating axons during the course of their continuous growth through the scar and the distal segment of the severed nerve. But today, now that we possess formulas of great analytic efficiency, such a hypothesis is absolutely untenable (2). Furthermore, it can be said that its new actual defenders reveal a state of mind favorable to compromise, as is shown by their many concessions to the doctrine of Waller, Ranvier, and Vanlair. I sincerely believe that, without the deplorable ostentation that consequently alienates so many good intentions and sterilizes or paralyzes so many talents, the conformity with the monogenistic theory would be absolute.

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(1) TELLO, myself and CASTRO nearly always employ 50 parts of pyridine to 100 parts of water and we transfer the pieces, after preliminary washing, into 96 % alcohol. After fixation in ammoniated alcohol the fusion in question is never present.

(2) MISKOLCKZY observed rightly that the quarrel between the partisans of continuity and those who believe in the doctrine of autogenesis arose from the difference in methods employed. I am convinced that if BETHE (today nearly the only defender of discontinous regeneration), before fixing his attitude on the question of regeneration had known the silver procedures, above all the formulas of reduced silver nitrate which makes it possible to obtain transparent, thick sections, he would have defended the Wallerian thesis. Despite this, in his later works he gives proofs of intellectual flexibility and scientific honesty by coming closer and closer to the theory of continuity. Nevertheless, he continues to defend as probable, in certain cases, a neoformation of nerve fibers, dependent

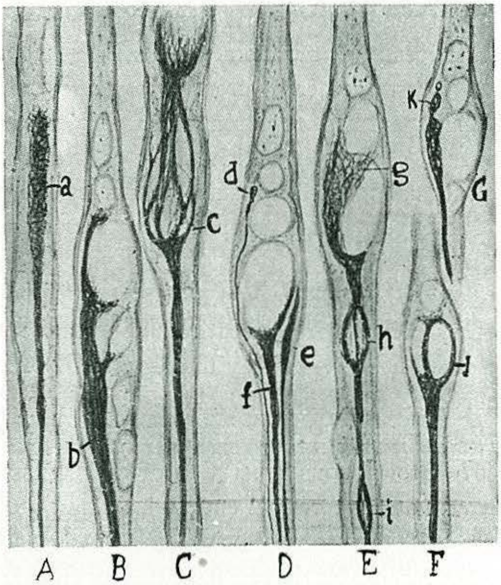

Fig. 67.—Details of the progression of the growth cones colliding with fat droplets from the peripheral ends of the fibers: *a, b, c, e,* cones of growth. This refutes categorically the hypothesis of autoregeneration (or discontinuous regeneration). Young cat sacrificed five hours after operation. The sprouts travel in the interior of the degenerated sheaths of Schwann.

In summary, every sectioned nerve regenerates its axons by means of sprouts from the central stump, which as Tello proved, cross the scar and assail the peripheral stump to reach the external sensory and

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at the peripheral end upon the cells of Schwann. Along with him Spielmeyer also, for the same technical reasons, continues to maintain his autogenetic attitude. See Bethe: Zur Theorie und Praxis der Verheilung durchtrennten Nerven. Volume in honor of Doctor Cajal, 1922. Consult B. Spielmeyer: *Zeitsch. f. Neurol. u. Psychiatrie,* Bd. 36, 1917. And his: Histopathologie des Nervensystem, Bd. I, 1922.

muscular terminations. Arriving to their destination, attracted no doubt by some substance (or physical influence unknown today) flowing from the nucleus of the terminal apparatus, the destroyed motor arborization moulds itself anew (1).

Concerning the hypothesis of *ensheathment* of the newly-formed axons through the scar, the old dispute between Bethe and his school and the new workers is reduced to a question of pure chronology. In the beginning the sprouts from the central stump emerge naked, and their ramifications course freely through the interstices of the connective tissue; but from the sixth or seventh day after the operation satellite

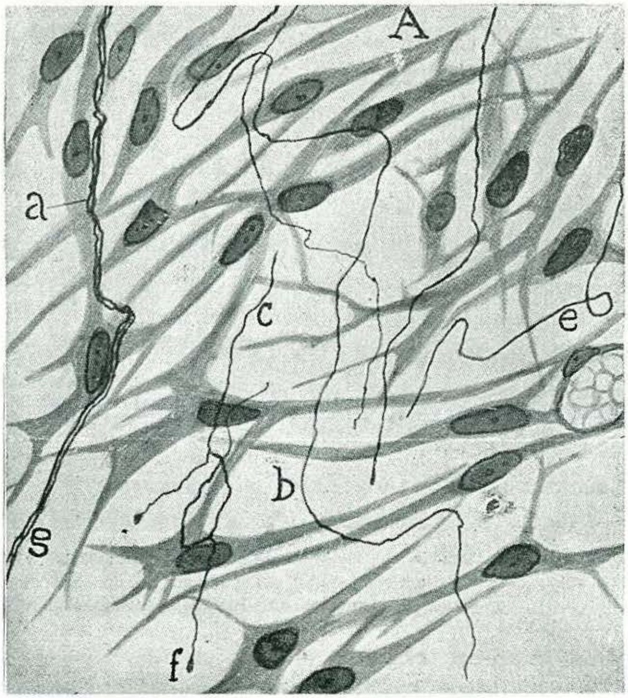

Fig. 68.—Scar near the central end of a cut nerve. The animal (cat) had been sacrificed 4 days after section: *A*, fibroblasts; *a*, fine exploring sprouts which follow fibroblasts of the beginning scar; *b*, *c*, *e*, other sprouts which travel freely through the plasma exudate. Note the absence of any protective envelope in the fibers arising from the central end.

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(1) Tello: Dégeneration et régeneration des plaques motrices, etc. *Trav. du Lab. de Rech. biol.* Tome V, 1907.

cells, which accompany them throughout their course and constitute a protective sheath, arise. Although they are not guiding cells (the *Leitzellen* of HELD or the *apotrophiques* of MARINESCO), even today the problem of the origin of these advential cells has not been resolved. The majority of neurologists are inclined to consider them as a progeny of SCHWANN's cells emigrating from the central stump of the mutilated nerve (I).

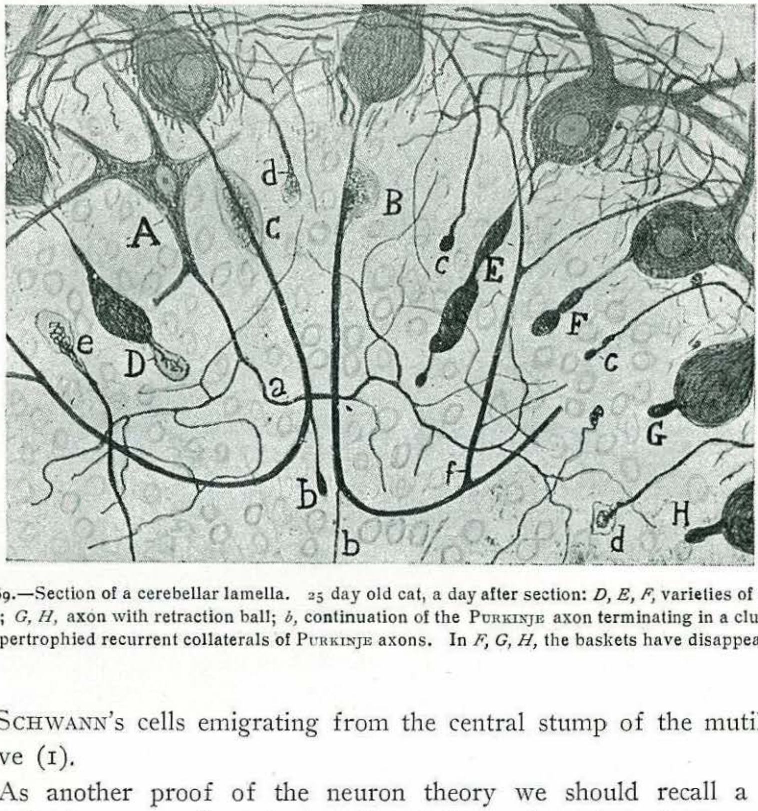

Fig. 69.—Section of a cerebellar lamella. 25 day old cat, a day after section: *D, E, F,* varieties of double clubs; *G, H,* axon with retraction ball; *b,* continuation of the PURKINJE axon terminating in a club; *c, d,* hypertrophied recurrent collaterals of PURKINJE axons. In *F, G, H,* the baskets have disappeared.

As another proof of the neuron theory we should recall a fact proved almost unanimously by all of the modern investigators: that anastomoses between the buds growing out of the central stump have never been found.

(I) Those who desire orientation in the study of these questions should consult the papers of BETHE, DUSTIN, LUGARO, PERRONCITO, MARINESCO, SPIELMEYER, TELLO, ROJAS, CASTRO, BOEKE, MISKOLCZY, etc., and mine particularly my work: Degeneración y Regeneración del sistema nervioso (an Englis translation was made by DOCTOR MAY).

C. **Regeneration in the nervous centers.**—Regeneration does not take place in the nervous centers. In favorable cases one may note phenomena of ramification, degeneration and neuronal metamorphosis, but never can the reëstablishment of the continuity of a sectioned neurite be observed. Very curious and significant in this regard is an experiment carried out by TELLO. When a piece of the peripheral end of a sectioned nerve is introduced into the cerebral wound of a rabbit (before the penetration of the sprouts), a regenerative capacity appears in the inactive neurites of the white substance. This demonstrates that the impotence of the central axis-cylinders to restore the peripheral segment is neither fatal nor irremediable, but that it is due, perhaps, to the absence of SCHWANN's cells in the process of rejuvenation (1).

Instead of this regenerative inactivity, the central axons show numerous degenerating transformations, the principal one of which is the formation, close or far away from the point of section, of a *ball* or *retraction club* (CAJAL) and the fragmentation of the axons in free balls ordinarily aligned close to the wound and below the initial collaterals. When the section is made in the region of the neurite from which these projections emanate, there is produced only a retraction club which is almost inevitably followed by the death of the mutilated neuron (fig. 69, D, G, F). Some of these phenomena, easily proved but neglected because they are troublesome for certain popular theories, have been confirmed by MISKOLCKZY (1924) and other impartial authors.

CHAPTER XIII

II.—ANATOMICOPATHOLOGICAL FACTS FAVORABLE TO THE NEURON THEORY. PERSISTENCE OF THE BASKETS AND OTHER PERICELLULAR TERMINATIONS, ETC.

The unity of the pathological reaction in neurons demands a clarifying restriction. Always when the disturbing causes (be they traumatic or chemicobiological) affect the integrity of a single neuron, the reaction is perfectly autonomous. But as the morbid processes usually present a diffuse character and act upon large or small foci, it is difficult to verify the unity of the pathological reaction, that is to say, the degeneration of the neuron and the integrity of the nervous arborizations with which it is in contact. It is not surprising that the neurons and the neurologlial and microglial cells with them are simultaneously affected either by microbial causes or by chemical conditions not always too well known.

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(1) TELLO: La influencia del neurotropismo en la regeneración de los centros nerviosos. *Trab. del Lab. de Invest. biol.* Tome IX, 1911.

Nevertheless, there are examples in which there appear here and there resistant groups of neurons or isolated cells affected very little or not at all, despite the extent of the lesion. I cite as examples *general paralysis,* FRIEDREICH's *disease, senile dementia, rabies,* necrotic foci produced by *emboli* or *thromboses,* alcoholic intoxication (ROSSI) or lead sals (VILLAVERDE), etc.

As a convincing and well known case let me mention the disappearance of the PURKINJE cells in *general paralysis* with maintenance of the basket and stellate cells of the molecular layer. This persistence, revealing the independence of the baskets and the cells they surround, can also be produced experimentally by sectioning the axons of the PURKINJE cells at the level of the granular layer or even below as is shown in figure 70 (1). This remarkable conservation of the baskets, despite the disappearance of the cells in connection with them has been described by SCHOB (2), and others, among them Río-HORTEGA (3), K. SCHAFFER, KINDBERG, CAJAL, NAGEOTTE, MARINESCO, SOMOZA, etc. The opposite phenomenon, that is, the destruction of the baskets, has been observed by BIELSCHOWSKY (4) in *amaurotic idiocy,* the histological pathology of which has been masterfully studied by K. SCHAFFER (5). BIELSCHOWSKY, etc. (figs. 69 and 71). Analogous cases of pathological dissociation between the PURKINJE cells and the climbing fibers have been noted. Authors such as WOLFF, HELD and others recognize anastomoses between the dendrites of neighboring PURKINJE cells (direct or indirect unions). Speaking against these authors are the processes of degeneration and the death of the axon (elongated portion of the cell), together with the conservation of the protoplasmic branches and the hypertrophy of the initial collaterals degenerative phenomenon which does not extend to the neighboring elements (UMBERTO ROSSI (1) and many other authors). Even more expressive is a lesion of these branches which I observed in trauma of the cerebellar white substance (2). The dendritic arborization is transformed and shrunken, acquiring the form of a rose bush (rose-like degeneration), but this curious retraction and metamorphosis does not spread to the adjoining cells (fig. 71).

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(1) CAJAL: Les phenomenes precoces de la régeneration neuronale dans le cervelet. *Trab. del Lab. de Invest. biol.* Tome IX, 1911.

(2) SCHOB: *Arb. aus der Deutsch Forsch-Anstalt f. Psychiatr. in München.* Bd. V, 1922.

(3) Río-HORTEGA: *Trab. del Lab. de Invest. biol.* Tome XII, 1914.

(4) BIELSCHOWSKY: Histopathologie der amaurotischen Idiotic, etc. *Journ. f. Psychol. u. Neurol.* Bd. XXVI, 1921.

(5) K. SCHAFFER: Tatsächliches und Hypothetisches aus der Histopathologie der infantil-amaurotischen Idiotie, 1912.

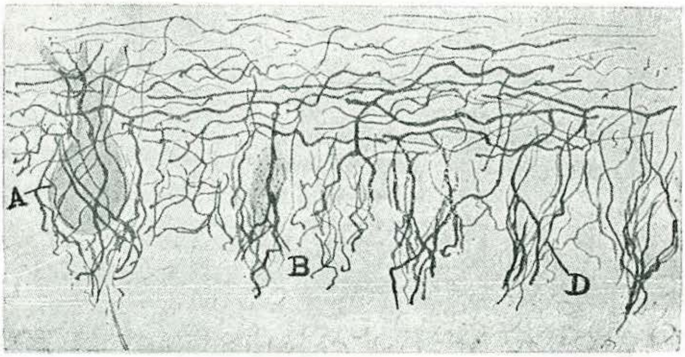

Fig. 70.—Cat of 25 days, 24 hours after operation: *A*, nearly normal PURKINJE cell; *B*, another in the process of atrophy and with a granular appearance; *D*, baskets which surround the cavity where the destroyed bodies of PURKINJE cells resided.

The fibers constituting a *basket* may also exhibit other important alterations which are significant for the thesis here defended. One of these is the hypertrophy and marked retraction of the terminal branches observed in many pathological states, particularly in general paralysis and dementia praecox (CAJAL). Another, also very expressive, consists of the total detachment of the nervous ramifications which withdraw themselves from the surrounded soma without leaving the slightest trace of comunicating neurofibrillar bridges. This abnormality, quite frequent in the pathological histology of the cerebellum, was first observed in the rabid dog by G. Izcara and me (1904). It is superflous to emphasize that both lesions constitute forceful arguments against the reticulum hypothesis.

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(1) U. ROSSI: Per la rigenerazione dei neuroni. *Trab, del Lab. de Invest. biol.* Tome VI, 1908.

(2) CAJAL: *Loc. cit. Trab. del Lab. de Invest. biol.* Tome IX, 1911.

In very limited trauma of the cerebrum, cerebellum, etc. (punctures with a needle or very fine scalpel), it is common to find degenerated cells which stand out among the neighboring uninjured pyramids. Indeed, some of the pyramids, the axons of which were severed below the collaterals, show a curious reaction: the initial collaterals hypertrophy (this occurs also in the Purkinje cells of the cerebellum) and here is encountered the surprising case in which cells of long axon are converted into neurons of short axon (1) (fig. 69, *c, d).*

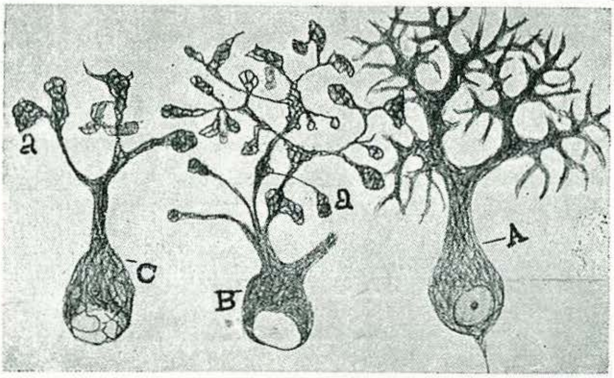

Fig. 71.—Purkinje cells of the cat of 25 days, sacrificed 2 hours after the traumatic lesion: *A*, normal corpuscle; *B, C,* cells in which the retracted dendrites terminate in reticulated clubs; *a*, terminal bulbs. The baskets have disappeared.

Similar proofs of histopathologic discontinuity can easily be obtained in the spinal cord and even in the medulla oblongata, although here well localized experimental lesions are more rare. Thus, recently, in our laboratory Castro and De Juan destroyed a part of the *ventral nucleus* of the acoustic nerve in the rabbit. I studied the cells of the nucleus of the *trapezoid body* using the BIELSCHOWSKY method and noted two days after the operation that the chalices of HELD became swollen, considerably hypertrophied and exhibited a granular degeneration while the surrounded neurons did not suffer any apparent injury and conserved their neurofibrils although these are not clearly visible.

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(1) Let us recall also the classical facts of Nissl's *chromatolysis* and the atrophy of the *motor nuclei* (Gudden's method) and finally, the slow destruction of motor neurons in Van Gehuchten's method, all very individualized neuronal lesions, following section or eradication of nerves.

By exhaustively consulting the bibliography one would find numerous examples similar to the ones mentioned.

Nevertheless, I cannot refrain from mentioning the experiments of LAWRENTJEW (1) and CASTRO (2), since they are modern and significant. The latter author has demonstrated that section of the afferent or preganglionic nerves of the sympathetic ganglia causes the disappearance of the pericellular and peridendritic arborizations after a rapid degenerative process (according to CASTRO, from seven to twenty-four hours after section of the ganglionic fibers), while the sympathetic cells of the vertebral chain persist, appearing normal six or seven days after the operation. In the experiments of LAWRENTJEW, section of the vagus produces degeneration of the pericellular plexuses of the cardiac sympathetic neurons, which remain normal 21 days after the operation. On the other hand, the nerve fibers arising from the autonomic cardiac ganglion, not being affected by the trauma, are preserved. Omitting here the doubtful normality, as I said before, of certain atypical nervous nests (NAGEOTTE and CAJAL) of the sensory and sympathetic corpuscles, a problem discussed in modern times by P. STÖHR (3), let us recall the fact that fibers of medullary origin in contact with certain sympathetic neurons do not produce the least alteration in these cells, at least for some time, when they degenerate. This result confirms the physiological interpretations of LANGLEY.

Obviously, such physiopathological persistence of the associated neurons is not endless. In reality, all the nervous elements in intimate connection suffer, following a lesion of the dynamically associated cells, and after months or years may undergo atrophy and degenerate because of disuse, provided that they do not have other connections capable of maintaining the functional activity.

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(1) LAWRENTJEW: Experimentelle-morphologische Studien über den feinerer Bau des autonomen Nervensystems. *Zeitsch f. mikros-anat. Forschung.* Bd. XVI, annee 1929.

(2) DE CASTRO: *Trab. du Lab. de Rech. biol.* Tome XXVI 1930.

(3) PH. STÖHR: Handbuch der mikroskopischen Anatomie des Menschen. Bd. IV, 1923. Herausg. v. W. Möllendorf.

These bibliographical notes are incomplete; to cite all of the authors who have stated these facts of anatomical dissociation would have required too much space.

In any case, the fact that the elements which have lost their principal connections persist for a greater or lesser time constitutes a strong indication of the anatomical discontinuity of nerve cells.

I do not wish to cite here physiological results too well known and incompatible with the theories of the diffuse nets of GOLGI, APATHY, BETHE and HELD, particularly the latter scholar's new concept concerning the structure of the gray substance, etc. Let us limit ourselves to declare that in accepting the most exaggerated syncitial hypotheses and extending them to the whole nervous system, all the local muscle reflexes, the specific sensory impressions (chromatic, acoustic, tactile, spatial, etc.) and actually everything that the physiologists, during 50 years of dogged and fruitful investigation, have taught us concerning localizations in the nervous centers is left without an explanation. We would fall, therefore, into chaos or hopeless nihilism unless, to avoid complete disaster to our better founded concepts of connections, we invoke DOGIEL's colonial hypothesis or one similar (anastomosis by Pleiades or nervous districts). But these intra-colonial anastomoses remain to be demonstrated and, even admitting them, they would clarify only a part of the physiological and pathological processes.

Perhaps microdissection combined with tissue culture will in time have the last word. But up to the present it has not been possible, at least as far as I know, to apply it to living nerve tissue of the centers. The general impression of those who have worked with microdissection is, as WOLLAND (1) declares in commenting upon the results of CHAMBERS' method (2) that the rarity of protoplasmic continuity (ephitelium) suggests

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(1) WOLLAND: Recent advances in Anatomy, 1927.

(2) CHAMBERS: The structure of the cells in tissues as revealed by microdissection. *The Journ. of Anatomy.* Vol. XXXV, 1925.

It is known that this scholar, by means of an ingenious microdissection apparatus associated with the method of tissue culture, has been able to evaluate lesions following cellular laceration with extremely fine needles. The effects provoked by the trauma are transmitted from some cells to other cells if protoplasmic bridges exist between them, but they are not transmitted if the

the idea that the bodies of animals, despite the almost metaphysical speculation of the adherents of the continuity theory, are actually composed of a cellular colony and that there is no necessity of changing anything in our histophysiological ideas as HELD and BOEKE maintain.

CONCLUSION.

I believe that I have adduced numerous and conclusive proofs favoring the neuron theory. To give them all in detail would have required a book. For me, as for all the investigators of the first epoch (KÖLLIKER, RETZIUS, VAN GEHUCHTEN, ATHIAS, DUVAL, MARINESCO, etc,) it is not a case of a more or less likely theory but rather a positive fact. What about doubtful fields observed in some cases and by means of certain techniques? I do not deny them. But the neurologist has the essential duty, common to all scientific workers, to distinguish the apparent from the real, the accidental technical fact from the preëxisting and general fact. At the hour of judgment we ought to depersonalize ourselves and forget seductive prejudices, whether ours own or others, seeing things, as GRACIÁN says, as if they were contemplated for the first time. And let us not fear the technical advances of the future because, if the facts have been observed well, they will endure although the interpretations may change.

I am neither exclusive nor dogmatic. I am proud of retaining a mental flexibility which is not afraid of corrections. Neuronal discontinuity, extremely evident in innumerable examples, could sustain some exceptions. I myself have mentioned some of them, for example those probably existing in the glands, vessels and intestines (my *interstitial neurons)*. Recently LAWRENTJEW (1) has confirmed

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opposite is the case. For example: in the epithelium of the skin lesions are propagated because of anastomoses; but the same is not true with epithelial or mesodermal cells without anastomoses.

(1) LAWRENTJEW: Über die Verbreitung der nervösen Elemente (einschliesslich der (interstitiellen Zellen) CAJAL's), etc. I differ with this scholar's interpretation which considers these cells as cells of SCHWANN because the cells of SCHWANN lack affinity for the stain of EHRLICH's method and silver chromate and because in addition to these characteristic they have a very special morphological structure. Perhaps he was not dealing with the same cells that I described in 1892 and 1893. See *Zeitsch. f. mikros-anat. Biol.-Wiss. biol. Abt.,* 1927.

anastomoses in the latter type cell. Neither would the existence of these unions by continuity in the coelenterates surprise me, although BOZLER recently has denied them, in preparations made with the EHRLICH method (1). This point demands an investigation whith modern methods.

Let us not fear, then, that the old, satisfying cellular conception of VIRCHOW may suffer grave damage by the attacks of the reticularists. The normal organism, being an association of relatively autonomus cells, always contains, as does a populous city, defective, deformed, abnormal and even gravely ill individuals alongside the normal. For this reason, which I have mentioned before and now insist upon, when dealing with morphology and neuronal connections we ought to abide by the law of the greatest numbers, that is to say, a rigorously statistical criterion.

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(1) BOZLER: Untersuchungen über das Nervensystem der Coelenteraten. I. Teil. Kontinuität oder Kontakt zwischen Nervenzellen. *Zeitsch. f. mikrosanat. Biol. Wiss. biol. Abt.*, 1927.

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